Innovative Food Technologies 14(1) (2026) 53-62

Iranian Research Organization for Science and Technology (IROST)

Innovative Food Technologies

journal homepage: https://jift.irost.ir

Effects of enzymatic pretreatment on phytochemical content, antioxidant, and pancreatic lipase inhibitory activities of *Solanum lasiocarpum* Dunal (terung asam) fruits

Andi Muhammad Nurfalahuddin Binhar[a], Mohammad Amil Zulhilmi Benjamin[b], Muhammad Naufal Qaweim Rushdy[a], Aniza Saini[a], Hasdian Mudin[a], Shiamala Devi Ramaiya[c], Rosnani Hasham [d] and Mohd Azrie Awang[a,e]

[a]*Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia*
[b]*Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400Kota Kinabalu, Sabah, Malaysia*
[c]*Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia, 97008 Bintulu Sarawak, Malaysia*
[d]*Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia*
[e]*Food Security Research Laboratory, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia*

ARTICLE INFO

Keywords:
Solanum lasiocarpum,
Crude yield,
Phytochemical content,
Antioxidant activity,
Pancreatic lipase inhibition,
Enzyme-assisted ultrasound
Extraction

ABSTRACT

Solanum lasiocarpum Dunal, locally known as terung asam, is an underutilised tropical fruit with recognised nutritional and bioactive potential. This study investigated enzyme-assisted ultrasound extraction of *S. lasiocarpum* fruit extract (SLFE) to improve crude yield (CY), phytochemical content, antioxidant activity, and pancreatic lipase inhibition. Enzymatic pretreatment using pectinase, cellulase, and protease was applied prior to ultrasound-assisted extraction. CY, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activities (DPPH, ABTS, and FRAP), and pancreatic lipase inhibition based on IC_{50} values were evaluated. Pectinase pretreatment produced the highest CY (35.80 ± 0.40%) and TFC (14.71 ± 0.16 mg RE/g) and consistently enhanced antioxidant activities across DPPH, ABTS, and FRAP assays. In contrast, cellulase-pretreated SLFE exhibited the strongest pancreatic lipase inhibition with an IC_{50} of 22.00 ± 0.12 µg/mL, followed by pectinase (24.20 ± 0.15 µg/mL), while protease showed the weakest inhibitory activity (62.05 ± 0.18 µg/mL). Cellulase pretreatment also yielded the highest TPC (78.24 ± 0.98 mg GAE/g). Pearson correlation analysis indicated significant relationships between phytochemical content and the measured bioactivities. Enzyme-assisted ultrasound extraction therefore represents an effective strategy for optimising functional compound recovery from SLFE, which may support its potential use in functional food and nutraceutical applications pending further biological validation.

1. Introduction

Solanum lasiocarpum Dunal, locally known as terung asam, is a traditional fruit widely consumed in Sarawak, Malaysia, and commonly used in local cuisines. Beyond its culinary importance, this species has been reported in traditional medicine in India for the management of fever, vomiting, sore throat, gonorrhoea, and female reproductive disorders. The berries and leaves have also been associated with anti-tussive, anti-asthmatic, anti-rheumatic, antiviral, anticancer, and spermicidal activities (Soon & Ding, 2021; Ibrahim et al., 2022). Recent studies by Saini et al. (2025a, 2025b) reported that *S. lasiocarpum* fruits contain diverse phenolic acids and flavonoids, with chlorogenic acids identified as major compounds associated with antioxidant activity and potential antidiabetic and anti-obesity effects. However, efficient recovery of these bioactive compounds remains challenging, as intact plant cell walls restrict solvent penetration and limit mass transfer during conventional extraction processes (Gil-Martín et al., 2022). Structural polysaccharides such as cellulose and pectin form complex barriers that entrap bioactive compounds within the plant matrix (Shahidi & Athiyappan, 2025). Enzyme-assisted extraction has therefore emerged as an effective approach to overcome these constraints by selectively hydrolysing specific cell wall components and enhancing compound release. When combined with ultrasound, which promotes cavitation and mechanical disruption, enzyme-assisted ultrasound extraction provides a synergistic strategy to improve extraction yield and bioactivity (Gil-Martín., 2022; Awang et al, 2023).

Obesity remains a major global health concern, with recent estimates indicating that in 2022 approximately one in eight people worldwide were living with obesity (WHO' to 'World Health Organization). One important therapeutic target in obesity management is pancreatic lipase, the primary enzyme responsible for hydrolysing dietary triglycerides into absorbable fatty acids and monoglycerides (Čolak & Pap, 2021). Inhibition of pancreatic lipase can reduce fat absorption and is therefore a widely recognised strategy for developing

* Corresponding author: ma.awang@ums.edu.my
10.22104/ift.2026.8118.2267
(Received: 08 January 2026, Received in revised form: 26 March 2026, Accepted: 06 April 2026
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

anti-obesity agents. Consequently, plant-derived bioactive compounds with antioxidant and lipase inhibitory properties have gained attention as potential alternatives to synthetic drugs, particularly for functional food and nutraceutical development (Machado et al., 2025; El-Saadony et al., 2025). Underutilised tropical fruits such as *S. lasiocarpum* are promising sources of such compounds due to their high phytochemical content, although their potential remains insufficiently explored because of limitations related to extraction efficiency and processing technologies (Villacís-Chiriboga et al., 2020).

Despite the recognised potential of enzyme-assisted ultrasound extraction, limited information is available regarding the comparative effects of different enzymes on crude yield (CY), antioxidant activity, and pancreatic lipase inhibition of *S. lasiocarpum* fruit extract (SLFE). Different enzymes target distinct structural components of the plant cell wall, and enzyme selection may therefore influence the recovery of phenolics, flavonoids, and lipase-inhibiting compounds in different ways. It was hypothesised that enzymes targeting specific cell wall polysaccharides, such as pectin and cellulose, would differentially affect the release of phytochemicals due to variations in plant cell wall composition. A systematic evaluation of enzyme specificity is therefore essential to optimise extraction efficiency and functional properties. This study aimed to investigate the effects of pectinase, cellulase, and protease pretreatment combined with ultrasound-assisted extraction on CY, phytochemical contents, antioxidant capacity, and pancreatic lipase inhibitory activity of SLFE. The findings are expected to provide insight into tailored extraction strategies and support further investigation of SLFE as a potential functional ingredient for functional food and nutraceutical applications.

2. Materials and methods

2.1. Raw materials

S. lasiocarpum fruits were collected from the Sibu area, Sarawak, Malaysia. The harvested fruits were thoroughly washed with tap water to remove adhering dirt and contaminants. The cleaned samples were placed on aluminium trays and dried in a ventilated oven equipped with a temperature controller and exhaust fan, maintaining an airflow rate of 1.4 m/s. Drying was carried out at 50 °C for 140 min following the optimal conditions reported by Jinin *et al.* (2024) with slight modifications. After drying, the samples were ground into a fine powder (<2 mm) using a grinder (EBM-9182, Elba, Borso del Grappa, Italy) and stored in airtight containers until further analysis.

2.2. Enzyme-assisted ultrasound extraction

The enzyme-assisted ultrasound extraction procedure was adapted from Ketemepi *et al.* (Ketemepi et al. (2024; 2025) with minor modifications. *S. lasiocarpum* fruits were cleaned to remove impurities, chopped into small pieces, and oven-dried at 50 °C until constant weight was achieved. The dried samples were subsequently ground into powder using a blender. The powdered sample (5 g) was mixed with 100 mL of 50 mM sodium acetate buffer (pH 5.0) to obtain a solid–liquid ratio of 1:20 (w/v). Enzymatic pretreatment was carried out to facilitate cell wall degradation and enhance the release of bioactive compounds. Pectinase (≥3000 U/mL, P2736), cellulase (≥0.3 U/mg solid, C1184), and acid-stable protease (≥3.5 U/mg solid, P5147) obtained from Sigma-Aldrich (St. Louis, MO, USA) were individually added at a concentration of 1% (w/w based on sample weight). The mixture was incubated for 2 h at 37 °C under continuous stirring to allow enzymatic hydrolysis of the plant matrix, thereby minimising thermal degradation of heat-sensitive phenolic compounds during enzymatic pretreatment.

Following enzymatic pretreatment, enzyme activity was terminated by heating the mixture at 85–95 °C for 10 min to inactivate the enzymes. The mixture was subsequently subjected to ultrasound-assisted extraction using an ultrasonic bath (CPX8800H, Branson, Brookfield, CT, USA) operating at a frequency of 44 kHz and power output of 250 W for 30 min at 40 °C. The resulting SLFE was filtered through Whatman No. 1 filter paper to remove insoluble residues. The filtrate was concentrated under reduced pressure using a rotary evaporator (Laborota 4000, Heidolph, Schwabach, Germany) at 50 °C to remove excess solvent. The concentrated SLFE was further dried in an oven at 50 °C for 24 h to obtain the crude extract. CY was calculated according to Equation (1).

$$\text{CY (\%)} = \frac{\text{Weight of crude extract (g)}}{\text{Weight of dried sample (g)}} \times 100 \quad (1)$$

2.3. Total phenolic content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method according to Ismail *et al.* (2020), with slight modifications. A reaction mixture comprising 500 µL of SLFE (1 mg/mL), 500 µL of Folin–Ciocalteu reagent, and 1.5 mL of 20% (w/v) sodium carbonate was adjusted to a final volume of 10 mL with distilled water. The mixture was thoroughly mixed and incubated in the dark for 2 h. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Lambda 25, PerkinElmer, Waltham, MA, USA). Gallic acid was used as the standard reference, and results were expressed as mg gallic acid equivalents (GAE) per g of dried extract, calculated from the calibration curve using Equation (2).

$$\text{TPC (mg GAE/g)} = \frac{c \times V}{m} \quad (2)$$

where c represents the concentration of the crude extract (mg/mL) obtained from the standard curve of TPC, V represents the solvent volume (mL), and m represents the sample mass (mg).

2.4. Total flavonoid content

Total flavonoid content (TFC) was determined using

the aluminium chloride colorimetric method described by Mudin *et al.* (2025), with slight modifications. A volume of 1 mL of SLFE (1 mg/mL) was mixed with 1 mL of 2% aluminium chloride and incubated for 15 min at 30 °C to allow formation of a flavonoid–aluminium complex. Absorbance was measured at 430 nm using a UV–Vis spectrophotometer. Rutin (0–100 mg/mL) was used as the calibration standard, and TFC was expressed as mg rutin equivalents (RE) per g of dried extract, calculated using Equation (3).

$$\text{TFC (mg RE/g)} = \frac{c \times V}{m} \quad (3)$$

where c represents the concentration of the crude extract (mg/mL) obtained from the standard curve of TFC, V represents the solvent volume (mL), and m represents the sample mass (mg).

2.5. DPPH radical scavenging assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was evaluated following the method described by Samary *et al.* (2025), with minor modifications. A reaction mixture consisting of 1 mL of SLFE (1 mg/mL) and 1 mL of 0.1 mM DPPH solution prepared in methanol was incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer. Trolox was used as the positive control. The percentage inhibition of DPPH radical scavenging activity was calculated using Equation (4).

$$\text{DPPH Inhibition Activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (4)$$

where $\text{Abs}_{\text{control}}$ is the absorbance of the control (DPPH solution without extract) and $\text{Abs}_{\text{sample}}$ is the absorbance of the sample (DPPH solution with extract).

2.6. ABTS radical scavenging assay

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity was determined using a modified method described by Rushdy *et al.* (2026). The ABTS radical cation was generated by reacting 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate, followed by incubation in the dark at room temperature for 24 h. The resulting solution was diluted with distilled water to obtain an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 °C prior to analysis. For the assay, 100 µL of SLFE (1 mg/mL) was mixed with 100 µL of ABTS reagent in a 96-well microplate and incubated for 5 min at room temperature. Absorbance was measured at 734 nm using a microplate reader (Multiskan SkyHigh, Thermo Fisher Scientific, Waltham, MA, USA). Trolox was used as the positive control, and the percentage inhibition of ABTS radical scavenging activity was calculated using Equation (5).

$$\text{ABTS Inhibition Activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (5)$$

where $\text{Abs}_{\text{control}}$ is the absorbance of the control (ABTS solution without extract) and $\text{Abs}_{\text{sample}}$ is the absorbance of the sample (ABTS solution with extract).

2.7. FRAP assay

The ferric reducing antioxidant power (FRAP) assay was performed following the method described by Benjamin *et al.* (2025), with slight modifications. The FRAP reagent was freshly prepared by mixing 38 mM sodium acetate buffer (pH 3.6), 20 mM ferric chloride, and 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) prepared in 40 mM hydrochloric acid at a ratio of 10:1:1. A volume of 20 µL of SLFE (1 mg/mL) was mixed with 180 µL of FRAP reagent and incubated at 37 °C for up to 30 min. Absorbance was measured at 593 nm using a UV–Vis spectrophotometer. Trolox was used as the standard reference, and FRAP values were expressed as mg Trolox equivalents (TE) per g of dried extract as calculated using Equation (6).

$$\text{FRAP (mg TE/g)} = \frac{c \times V}{m} \quad (6)$$

where c represents the concentration of the crude extract (mg/mL) obtained from the standard curve of FRAP, V represents the solvent volume (mL), and m represents the sample mass (mg).

2.8. Pancreatic lipase inhibition assay

Pancreatic lipase inhibitory activity was evaluated following the method described by Estribillo *et al.* (2022). A stock solution (100 µg/mL) was prepared by dissolving 1 mg of SLFE in 10 mL of 100 mM phosphate buffer (pH 7.2) containing 0.5% (v/v) Triton X-100. Working solutions at concentrations of 20, 30, 40, 50, 60, and 70 µg/mL were prepared from the stock solution. Orlistat dissolved in dimethyl sulfoxide was used as the positive control. For the assay, 25 µL of SLFE or orlistat was mixed with 50 µL of pancreatic lipase solution. Subsequently, 100 µL of phosphate buffer and 25 µL of *p*-nitrophenyl butyrate (PNPB) substrate were added to each well of a 96-well microplate. The plate was incubated at 37 °C for 30 min. Lipase activity was determined by measuring the release of *p*-nitrophenol at 400 nm using a microplate reader. The percentage inhibition of pancreatic lipase activity was calculated using Equation (8).

$$\text{Lipase Inhibition Activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (8)$$

where $\text{Abs}_{\text{control}}$ is the absorbance of the control (Lipase solution without extract) and $\text{Abs}_{\text{sample}}$ is the absorbance of the sample (Lipase solution with extract).

2.9. Statistical analysis

All experiments were conducted in triplicate (n = 3), and results were expressed as mean ± standard deviation.

Statistical analyses were performed using IBM SPSS Statistics (Version 28). One-way analysis of variance (ANOVA) was used to determine significant differences between enzyme pretreatments, followed by Tukey's test when significant effects were observed at $p < 0.05$. Bar graph and regression analyses were employed to determine IC_{50} values, defined as the concentration required to inhibit 50% of DPPH and ABTS radicals as well as pancreatic lipase activity, using Microsoft Excel (2019).

3. Results and discussion

3.1. Effect of enzymatic pretreatment on CY of SLFE

CY of SLFE following enzymatic pretreatment is presented in Fig. 1. Among the enzymes evaluated, pectinase pretreatment resulted in the highest CY (35.80 ± 0.40%), which was significantly higher than those obtained using cellulase (12.74 ± 0.30%) and protease (10.27 ± 0.30%) ($p < 0.05$). These results demonstrate that enzyme type plays a critical role in determining extraction efficiency. The markedly higher CY observed for pectinase-treated SLFE indicates that this enzyme is particularly effective in enhancing solubilisation and mass transfer during extraction, thereby promoting greater recovery of extractable components from the plant matrix.

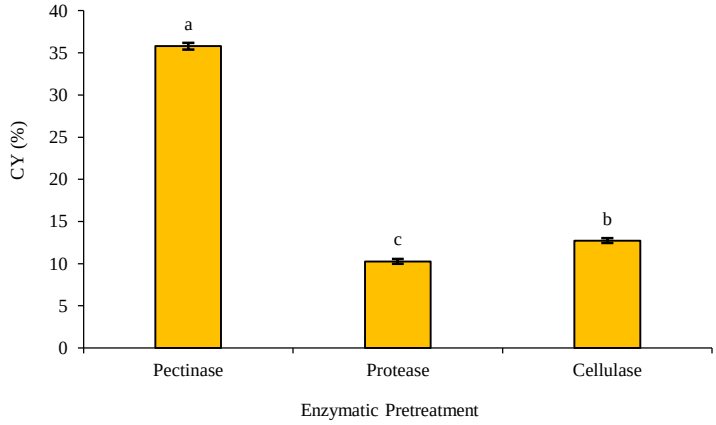
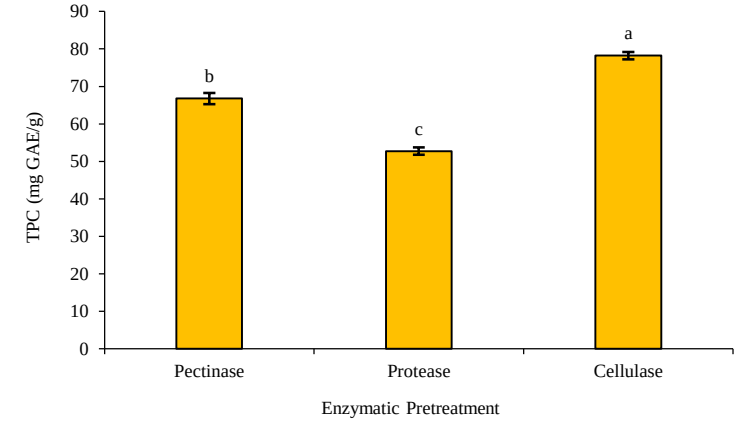


Fig. 1. Effects of enzymatic pretreatment on CY of SLFE. Values are expressed as mean ± standard deviation (n = 3). Different letters indicate significant differences determined by one-way ANOVA followed by Tukey's test ($p < 0.05$).

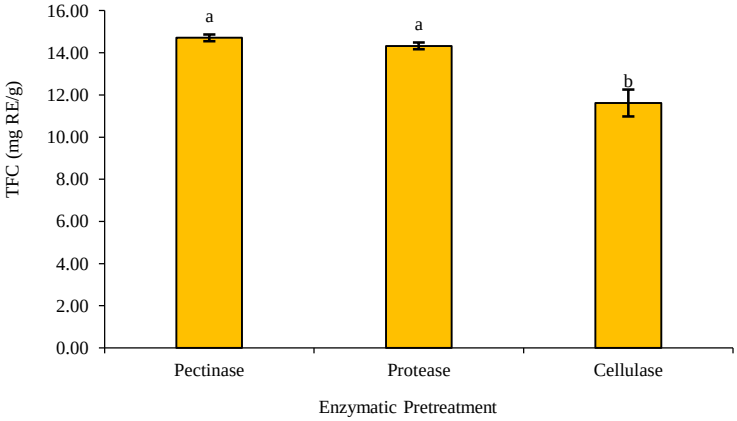
The superior CY obtained with pectinase pretreatment can be attributed to its specific action on pectic substances located in the middle lamella and primary cell wall (Haile & Ayele, 2022). Degradation of pectin reduces cell-to-cell adhesion and softens plant tissues, which facilitates solvent penetration and enhances the release of soluble compounds into the extraction medium. Enzymatic hydrolysis of pectin-rich matrices also improves mass transfer by increasing solubility and diffusion of phytochemicals into the extraction medium (Dixit et al., 2025). In addition, enzymatic cleavage of glycosidic bonds may induce transformations such as deglycosylation of flavonoid glycosides, producing aglycone forms with altered solubility and bioactivity (Lee et al., 2025). In contrast, cellulase hydrolyses cellulose microfibrils, which constitute the structural framework of the plant cell wall. Although this process disrupts the rigid polysaccharide network and enhances cell wall permeability, the resulting release of soluble compounds may be less extensive than that obtained through pectin degradation (Wu et al., 2026). Protease pretreatment produced the lowest CY, reflecting the limited contribution of protein hydrolysis to overall extract solubilisation, as most extractable compounds in SLFE are associated with carbohydrate-rich cell wall components rather than protein matrices (Vogelsang-O'Dwyer et al., 2022). Overall, these findings demonstrate that enzymatic pretreatment significantly influences extraction efficiency, and that pectinase is the most effective enzyme for maximising CY of SLFE. The results also highlight the importance of enzyme specificity in targeting different structural components of plant tissues to enhance extraction performance.

3.2. Effect of enzymatic pretreatment on TPC and TFC of SLFE

As shown in Fig. 2a., TPC of SLFE varied significantly among the different enzymatic pretreatments ($p < 0.05$). Cellulase pretreatment resulted in the highest TPC (78.24 ± 0.98 mg GAE/g), followed by pectinase (66.80 ± 1.50 mg GAE/g), while protease pretreatment produced the lowest TPC (52.75 ± 0.98 mg GAE/g). These results demonstrate that enzymatic pretreatment significantly influenced phenolic recovery, with cellulase showing the greatest effectiveness in enhancing TPC of SLFE. Fig. 2b. shows the TFC of SLFE following enzymatic pretreatment. Pectinase pretreatment resulted in the highest TFC (14.71 ± 0.16 mg RE/g), which was comparable to that obtained with protease pretreatment (14.32 ± 0.16 mg RE/g), while cellulase pretreatment produced a significantly lower TFC (11.62 ± 0.64 mg RE/g). Statistical analysis confirmed a significant difference between cellulase and the other enzyme treatments ($p < 0.05$), whereas no significant difference was observed between pectinase and protease ($p > 0.05$).



(a)



(b)

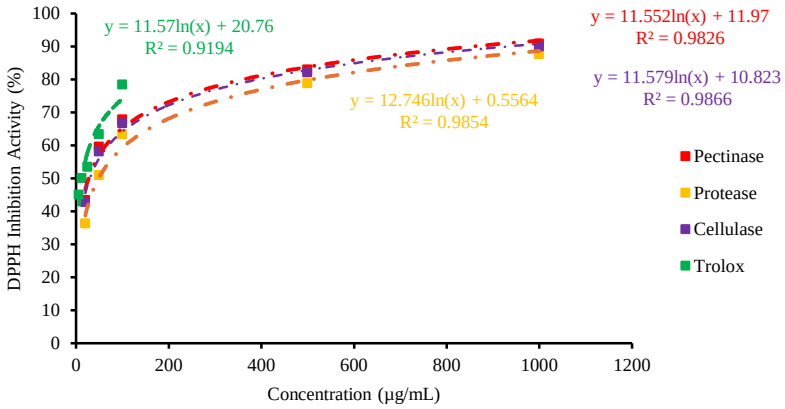
Fig. 2. Effects of enzymatic pretreatment on (a) TPC and (b) TFC of SLFE. Values are expressed as mean ± standard deviation (n = 3). Different letters indicate significant differences determined by one-way ANOVA followed by Tukey's test ($p < 0.05$).

Cellulase-pretreated SLFE exhibited the highest TPC, indicating that disruption of cellulose microfibrils effectively facilitates the release of phenolic compounds that are entrapped within or associated with cellulose-rich regions of the cell wall. Increased cell wall permeability following cellulose hydrolysis enhances solvent penetration and improves diffusion of phenolic compounds into the extraction medium (Wu et al., 2026; Mardiah et al full stop, 2025). Pectinase pretreatment resulted in a moderate increase in TPC, reflecting its primary action on pectic-rich regions of the cell wall rather than on cellulose-associated phenolics (Haile & Ayele, 2022; Dixit et al., 2025). In addition, interactions between released phenolic compounds and pectin-derived polysaccharides may influence the physicochemical behaviour of phenolics, including solubility and stability, which can affect their recovery during extraction (Hosseini et al, 2020). Protease pretreatment produced the lowest TPC, consistent with the relatively limited association between phenolic compounds and protein matrices in plant tissues (Vogelsang-O'Dwyer et al., 2022). Similar observations have been reported in protein-rich materials such as canola oil press cakes, where protease pretreatment reduced phenolic retention within protein complexes (Tian et al., 2023).

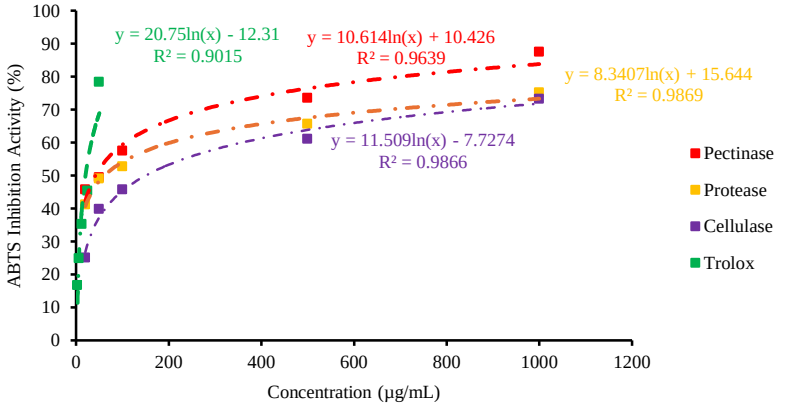
In contrast to the phenolic profile, TFC was highest in pectinase-pretreated SLFE and was comparable to that obtained with protease pretreatment, while cellulase resulted in significantly lower flavonoid recovery. This trend reflects the preferential localisation of many flavonoids in pectin-rich regions of the cell wall or within intracellular compartments rather than in cellulose-dominated structures (Shi et al., 2024). Degradation of pectin reduces tissue rigidity and promotes solvent accessibility, facilitating the release of flavonoids that are commonly localised in vacuoles or associated with pectic polysaccharides (Dixit et al., 2025). Furthermore, the aluminium chloride colorimetric method selectively detects flavonols and flavones through complex formation with hydroxyl groups, and enzymatic pretreatment may enhance flavonoid detectability by promoting partial hydrolysis of flavonoid glycosides into more reactive aglycone forms due to auxiliary enzymatic activities in commercial preparations (Chang et al., 2002; Wen et al., 2025). These findings indicate that enzyme specificity strongly influences phytochemical recovery from SLFE, with cellulase favouring phenolic extraction and pectinase providing greater efficiency for flavonoid recovery under ultrasound-assisted conditions.

3.3. Effect of enzymatic pretreatment on antioxidant activity of SLFE

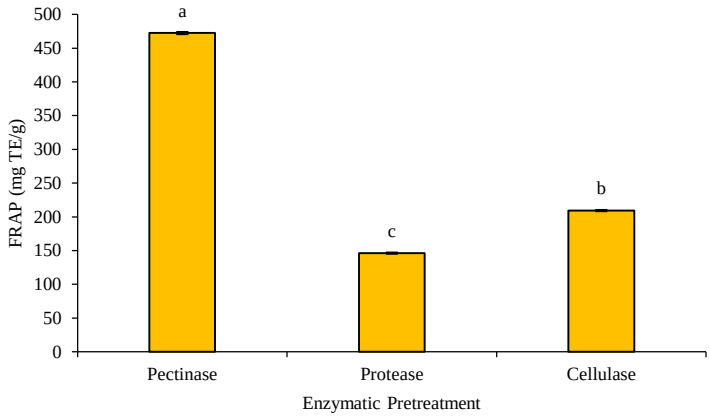
Fig. 3a. shows the DPPH radical scavenging activity of SLFE following enzymatic pretreatment. All enzyme-pretreated SLFE exhibited strong DPPH scavenging activity, exceeding 87%. Statistical analysis confirmed significant differences among enzyme treatments ($p < 0.05$). Pectinase-treated SLFE demonstrated the highest scavenging activity, followed closely by cellulase, while protease showed slightly lower activity. This trend was supported by IC_{50} values, where pectinase-treated SLFE exhibited the lowest IC_{50} (26.88 ± 0.14 µg/mL), followed by cellulase (29.45 ± 0.17 µg/mL) and protease (48.37 ± 0.18 µg/mL). Trolox, used as the positive control, showed a substantially lower IC_{50} (12.51 ± 0.08 µg/mL), confirming higher radical scavenging potency compared with SLFE. The ABTS radical scavenging activity of SLFE is presented in Fig. 3b. All enzyme-pretreated SLFE exhibited strong ABTS scavenging activity, exceeding 73%. Enzyme treatments significantly affected the ABTS radical scavenging activity of SLFE ($p < 0.05$). Pectinase-treated SLFE showed the highest ABTS scavenging activity, followed by protease, while cellulase resulted in the lowest activity. Consistent with this pattern, pectinase-treated SLFE exhibited the lowest ABTS IC_{50} value (41.55 ± 0.17 µg/mL), followed by protease (61.51 ± 0.21 µg/mL) and cellulase (150.87 ± 0.25 µg/mL). Trolox showed a markedly lower IC_{50} (20.14 ± 0.09 µg/mL), indicating stronger ABTS radical scavenging efficiency relative to SLFE. These results demonstrate that enzymatic pretreatment significantly influenced ABTS antioxidant capacity of SLFE. Fig. 3c. illustrates the FRAP of SLFE following enzymatic pretreatment. Pectinase pretreatment resulted in the highest FRAP value (472.54 ± 1.54 mg TE/g), which was significantly higher than values obtained with cellulase (209.33 ± 0.81 mg TE/g) and protease (146.11 ± 1.01 mg TE/g) ($p < 0.05$). Overall, these findings indicate that enzymatic pretreatment markedly affected antioxidant capacity of SLFE, with pectinase consistently enhancing both radical scavenging and reducing power across DPPH, ABTS, and FRAP assays.



(a)



(b)



(c)

Fig. 3. Effects of enzymatic pretreatment on (a) DPPH, (b) ABTS, and (c) FRAP antioxidant activities of SLFE. Values are expressed as mean ± standard deviation (n = 3). Different letters indicate significant differences determined by one-way ANOVA followed by Tukey's test ($p < 0.05$).

The antioxidant activity of SLFE varied significantly among the enzymatic pretreatments, reflecting differences in the release of phenolic and flavonoid compounds. The higher DPPH radical scavenging activity observed in pectinase-treated SLFE is consistent with the enhanced release of flavonoids and other polyphenolic compounds following degradation of pectin-rich cell wall regions. These compounds possess multiple hydroxyl groups that act as efficient hydrogen and electron donors, thereby contributing to radical neutralisation (Gulcin & Alwasel, 2023). A similar trend was observed for ABTS radical scavenging activity, where pectinase pretreatment produced the strongest antioxidant response. The enhanced activity may be attributed to improved solvent penetration and increased accessibility of polyphenols following enzymatic disruption of pectin-rich structures. Polyphenolic compounds containing conjugated aromatic rings and multiple hydroxyl groups exhibit strong reactivity towards ABTS radicals through both hydrogen atom and electron transfer mechanisms (Stachelska et al., 2025). In contrast, cellulase and protease pretreatments exhibited moderate antioxidant responses, which is consistent with the comparatively lower release of flavonoids and other antioxidant phytochemicals. The FRAP assay further supported the superior antioxidant performance of pectinase-treated SLFE. This assay evaluates antioxidant capacity through a single electron transfer mechanism in which antioxidants reduce ferric ions from Fe^{3+} to Fe^{2+} (Hsieh & Rajashekaraiah, 2021). The higher FRAP values obtained following pectinase pretreatment suggest enhanced release of polyphenolic compounds with strong reducing capacity (Shi et al., 2024). However, ferric reducing activity may also reflect the contribution of other phytochemicals, including phenolic acids and related secondary metabolites present in the extract.

Similar observations have been reported in previous enzyme-assisted extraction studies, where enzymatic degradation of cell wall polysaccharides enhances the release of phenolic compounds and improves antioxidant performance (Nunes et al., 2025). Veer et al. (2026) demonstrated that pectinase-assisted extraction significantly enhanced the yield and bioactive quality of red dragon fruit juice by degrading pectin-rich mucilage that restricts mass transfer, resulting in higher juice yield (72.25%), TPC (192.72 mg GAE/100 mL), and antioxidant activity (73.87% DPPH inhibition) compared with non-enzymatic extraction. Comparable improvements in antioxidant activity following pectinase treatment have also been reported for apricot juice, where increased release of phenolics and flavonoids enhanced DPPH and ABTS radical scavenging activities (Bashir et al., 2021). Similar improvements in bioactive compound recovery have also been reported for *Lycium ruthenicum*, where pectinase-assisted extraction (1.5% pectinase at 50 °C for 30 min) produced higher extraction yield, total anthocyanin content, TPC than other extraction approaches (Vidana Gamage & Choo, 2023). Overall, the consistent ranking observed across DPPH, ABTS, and FRAP assays indicates that pectinase pretreatment most effectively enhances antioxidant mechanisms associated with hydrogen- and electron-donating phytochemicals in SLFE.

3.4. Effect of enzymatic pretreatment on lipase inhibitory activity of SLFE

Pancreatic lipase inhibitory activity of SLFE following enzymatic pretreatment was evaluated based on IC_{50} values obtained from the tested concentration range of 20–70 µg/mL, with orlistat used as the positive control. As shown in Fig. 4., cellulase-pretreated SLFE exhibited the lowest IC_{50} value (22.00 ± 0.12 µg/mL), indicating the strongest lipase inhibitory activity among the enzyme treatments. This was followed by pectinase-pretreated SLFE with an IC_{50} of' between value and 24.20± 0.15 µg/mL, while protease-pretreated SLFE showed the weakest inhibitory activity with the highest IC_{50} value (62.05 ± 0.18 µg/mL). Orlistat displayed a markedly lower IC_{50} (0.82 ± 0.03 µg/mL), confirming its superior inhibitory potency compared with SLFE. These results demonstrate that enzymatic pretreatment significantly influenced the lipase inhibitory efficacy of SLFE within the tested concentration range ($p < 0.05$).

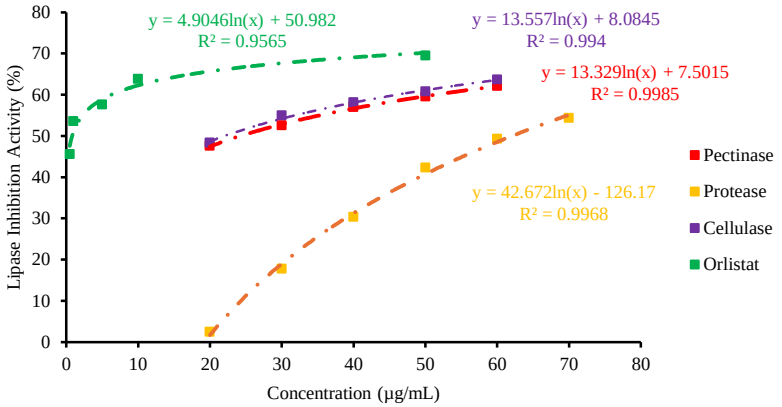


Fig. 4. Pancreatic lipase inhibitory activity of orlistat and enzyme-pretreated SLFE at different concentrations. Values are expressed as mean ± standard deviation (n = 3).

The strongest pancreatic lipase inhibitory activity observed for cellulase-pretreated SLFE suggests that disruption of cellulose-rich structural components may enhance the release of phenolic compounds associated with lipase inhibition. Increased cell wall permeability following cellulase hydrolysis facilitates the diffusion of phenolic constituents into the extraction medium, which may interfere with pancreatic lipase activity by limiting lipid hydrolysis processes (Wu et al., 2026). Pectinase-pretreated SLFE exhibited slightly lower inhibitory activity, although degradation of pectin-rich regions can also promote the release of bioactive compounds, including flavonoids that have been reported to interact with pancreatic lipase (Haile & Ayele, 2022; Li et al, 2022). In contrast, protease pretreatment produced the weakest lipase inhibitory activity, which is consistent with the limited contribution of protein-associated components to lipase inhibition in plant extracts (Vogelsang-O'Dwyer, 2022). These results indicate that enzymatic pretreatment significantly influences the anti-lipase potential of SLFE. The stronger inhibitory activity observed in cellulase-pretreated extracts may be associated with the greater release of phenolic compounds, whereas pectinase treatment may preferentially enhance flavonoid recovery. Overall, these findings suggest that the anti-lipase activity of SLFE is influenced by the combined contribution of multiple phytochemical constituents rather than a single compound class.

3.5. Correlation analysis of TPC and TFC with antioxidant and lipase inhibitory activities of SLFE

Pearson correlation analysis was performed to evaluate the relationships between TPC, TFC, antioxidant activities (DPPH, ABTS, and FRAP), and lipase inhibition, as presented in Table 1. TPC exhibited a significant positive correlation with DPPH radical scavenging activity (r = 0.725), indicating that higher phenolic content was associated with stronger DPPH antioxidant activity. Conversely, TPC showed a significant negative correlation with ABTS activity (r = −0.836) and lipase inhibition (r = −0.911), suggesting that increased phenolic concentration corresponded to lower ABTS scavenging capacity and lipase inhibitory activity. TFC demonstrated a strong negative correlation with DPPH activity (r = −0.973), indicating an inverse relationship between flavonoid content and DPPH radical scavenging. However, TFC showed weak and non-significant correlations with ABTS (r = 0.287), and lipase inhibition (r = 0.430). Furthermore, neither TPC nor TFC showed a significant association with FRAP activity (TPC: r = 0.239; TFC: r = 0.427), indicating that ferric reducing capacity may be influenced by other phytochemical constituents in the extract. Overall, these results suggest that phenolic and flavonoid compounds contribute differently to antioxidant mechanisms and lipase inhibition in SLFE.

Table 1. Pearson correlation coefficients of TPC and TFC with antioxidant activities and lipase inhibitory activity of SLFE.

Variables	DPPH	ABTS	FRAP	Lipase
TPC	0.725*	−0.836**	0.239	−0.911**
TFC	−0.973**	0.287	0.427	0.430

Values represent Pearson correlation coefficients (r). p values were calculated using a two-tailed test. $p < 0.05$ (*), $p < 0.01$ (**).

4. Conclusions

This study demonstrates that enzyme-assisted ultrasound extraction is an effective approach for enhancing CY and bioactivity of SLFE. Enzymatic pretreatment significantly influenced extraction efficiency, antioxidant capacity, and pancreatic lipase inhibitory activity, confirming the importance of enzyme specificity in optimising extraction outcomes. Pectinase pretreatment produced the highest CY and TFC and consistently enhanced antioxidant performance across DPPH, ABTS, and FRAP assays. In contrast, cellulase pretreatment resulted in the highest TPC and exhibited the strongest pancreatic lipase inhibitory activity based on IC_{50} values, while protease pretreatment demonstrated comparatively limited effectiveness across most evaluated parameters. Overall, these findings indicate that targeted enzymatic disruption of specific plant cell wall components can selectively enhance the release of bioactive compounds associated with distinct functional properties, as supported by the observed correlations

between phytochemical content and bioactivities.

From a practical perspective, the use of pectinase-assisted ultrasound extraction offers a promising strategy for improving the recovery of bioactive compounds from underutilised fruits such as *S. lasiocarpum*, which may contribute to future functional food and nutraceutical development following further biological and clinical evaluation. However, the present study was limited to *in vitro* antioxidant and lipase inhibitory assays and relied on global spectrophotometric methods for phytochemical quantification, without chromatographic profiling of individual compounds. Techniques such as high-performance liquid chromatography (HPLC) or liquid chromatography–mass spectrometry (LC–MS) were not employed in the present study, which limits detailed mechanistic interpretation of the specific bioactive constituents responsible for the observed activities. Future studies should therefore focus on detailed phytochemical profiling, identification of active constituents, and validation of biological effects through *in vivo* or clinical investigations to further substantiate the functional potential of SLFE.

Acknowledgments

This study was supported by the Ministry of Higher Education Malaysia through the Fundamental Research Grant Scheme Early Career (FRGS-EC/1/2024/TK05/UMS/02/4).

References

Awang, M. A., Nik Mat Daud, N. N. N., Mohd Ismail, N. I., Abdullah, F. I., & Benjamin, M. A. Z. (2023). A review of *Dendrophthoe pentandra* (mistletoe): Phytomorphology, extraction techniques, phytochemicals, and biological activities. *Processes*, *11*(8), 2348. https://doi.org/10.3390/pr11082348

Bashir, O., Hussain, S. Z., Gani, G., Jan, N., Rather, A. H., Reshi, M., & Amin, T. (2021). Evaluating the physicochemical and antioxidant characteristics of apricot juice prepared through pectinase enzyme-assisted extraction from Halman variety. *J. Food Meas. Charact.*, *15*(3), 2645–2658. https://doi.org/10.1007/s11694-021-00833-w

Benjamin, M. A. Z., Vincent, J., Binsali, H. N., Mus, A. A., Awang, M. A., & Rusdi, N. A. (2025). Volatile components, antibacterial and antioxidant activities of komburongoh (*Acorus calamus* L.) essential oils as potential medicinal herbs from Sabah, Malaysia. *Borneo J. Resour. Sci. Technol.*, *15*(1), 116–129. https://doi.org/10.33736/bjrst.6872.2025

Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.*, *10*(3), 178–182. https://doi.org/10.38212/2224-6614.2748

Čolak, E., & Pap, D. (2021). The role of oxidative stress in the development of obesity and obesity-related metabolic disorders. *J. Med. Biochem.*, *40*(1), 1–9. https://doi.org/10.5937/jomb0-24652

Dixit, S. S., Muruganandam, L., & Ganesh Moorthy, I. (2025). Pectin from fruit peel: A comprehensive review on various extraction approaches and their potential applications in pharmaceutical and food industries. *Carbohydr. Polym. Technol. Appl.*, *9*, 100708. https://doi.org/10.1016/j.carpta.2025.100708

El-Saadony, M. T., Saad, A. M., Mohammed, D. M., Alkafaas, S. S., El-Mageed, T. A. A., Fahmy, M. A., Ahmed, A. E., Algopishi, U. B., Abu-Elsaoud, A. M., Mosa, W. F. A., AbuQamar, S. F., & El-Tarabily, K. A. (2025). Plant bioactive compounds: Extraction, biological activities, immunological, nutritional aspects, food application, and human health benefits—A comprehensive review. *Front. Nutr.*, *12*, 1659743. https://doi.org/10.3389/fnut.2025.1659743

Estribillo, A. G. M., Gaban, P. J. V., Rivadeneira, J. P., Villanueva, J. C., Torio, M. A. O., & Castillo-Israel, K. A. T. (2022). Evaluation of *in vitro* lipid-lowering properties of 'Saba' banana [*Musa acuminata* x *balbisiana* (BBB group) 'Saba'] peel pectin from different extraction methods. *Malays. J. Nutr.*, *28*(1), 65–77. https://doi.org/10.31246/MJN-2020-0121

Gil-Martín, E., Forbes-Hernández, T., Romero, A., Cianciosi, D., Giampieri, F., & Battino, M. (2022). Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products. *Food Chem.*, *378*, 131918. https://doi.org/10.1016/j.foodchem.2021.131918

Gulcin, İ., & Alwasel, S. H. (2023). DPPH radical scavenging assay. *Processes*, *11*(8), 2248. https://doi.org/10.3390/pr11082248

Haile, S., & Ayele, A. (2022). Pectinase from microorganisms and its industrial applications. *Sci. World J.*, *2022*, 1881305. https://doi.org/10.1155/2022/1881305

Hosseini, S., Parastouei, K., & Khodaiyan, F. (2020). Simultaneous extraction optimization and characterization of pectin and phenolics from sour cherry pomace. *Int. J. Biol. Macromol.*, *158*, 911–921. https://doi.org/10.1016/j.ijbiomac.2020.04.241

Hsieh, C., & Rajashekaraiah, V. (2021). Ferric reducing ability of plasma: A potential oxidative stress marker in stored plasma. *Acta Haematol. Pol.*, *52*(1), 61–67. https://doi.org/10.5603/AHP.2021.0009

Ibrahim, N. F., Zakaria, N. A., & Aris, F. (2022). Phytochemistry and biological activity of terung asam, indigenous fruit-vegetables of Sarawak - A review. *J. Sustain. Sci. Manag.*, *17*(2), 270–285. https://doi.org/10.46754/jssm.2022.02.019

Ismail, M. F., Ramaiya, S. D., Zakaria, M. H., Mohd Ikhsan, N. F., & Awang, M. A. (2020). Mineral content and phytochemical properties of selected *Caulerpa* species from Malaysia. *Malays. J. Sci.*, *39*(3), 115–131. https://doi.org/10.22452/mjs.vol39no3.10

Jinin, N. A. Z., Benjamin, M. A. Z., & Awang, M. A. (2024). Drying kinetics and quality assessment of noodles from *Piper sarmentosum* Roxb. (kaduk) leaves. *Malays. J. Fund. Appl. Sci.*, *20*(4), 835–851. https://doi.org/10.11113/mjfas.v20n4.3519

Ketemepi, H. K., Awang, M. A. Bin, Pindi, W., Narayanan, A. S., & M. N., N. Q. I. (2025). Data-driven high-performance liquid chromatography method for the simultaneous analysis of disodium guanylate and disodium inosinate in mushrooms. *Data Brief*, *62*, 111847. https://doi.org/10.1016/j.dib.2025.111847

Ketemepi, H. K., Awang, M. A. Bin, Seelan, J. S. S., & Mohd Noor, N. Q. I. (2024). Extraction process and applications of mushroom-derived protein hydrolysate: A comprehensive review. *Future Foods*, *9*, 100359. https://doi.org/10.1016/j.fufo.2024.100359

Lee, C.-D., Kim, E.-A., Rho, H. S., & Lee, S. (2025). Enzymatic transformation of secondary metabolites in

Abeliophyllum distichum extract by Viscozyme® L treatment. *Chemosensors*, *13*(9), 331. https://doi.org/10.3390/chemosensors13090331

Li, N., Liu, X., Zhang, J., Lang, Y.-Z., Lu, L., Mi, J., Cao, Y.-L., Yan, Y.-M., & Ran, L.-W. (2022). Preventive effects of anthocyanins from *Lycium ruthenicum* Murray in high-fat diet-induced obese mice are related to the regulation of intestinal microbiota and inhibition of pancreatic lipase activity. *Molecules*, *27*(7), 2141. https://doi.org/10.3390/molecules27072141

Machado, M., Bautista-Hérnandez, I., Gómez-García, R., Silva, S., & Costa, E. M. (2025). Bioactive food proteins: Bridging nutritional and functional benefits with sustainable protein sources. *Foods*, *14*(17), 3035. https://doi.org/10.3390/foods14173035

Mardiah, M., Fulazzaky, M. A., Rahmawati, S. I., & Fulazzaky, M. (2025). Enzyme-assisted extraction of bioactive compounds from roselle calyx. *Next Res.*, *2*(3), 100536. https://doi.org/10.1016/j.nexres.2025.100536

Mudin, H., Benjamin, M. A. Z., Rusdi, N. A., & Awang, M. A. (2025). Optimisation of fruit vinegar process from *Baccaurea lanceolata* (Miq.) Müll.Arg using central composite design with antioxidant and antimicrobial potential. *Jurnal Teknologi*, *87*(5), 987–998. https://doi.org/10.11113/jurnalteknologi.v87.23406

Nunes, A. R., Alves, G., Falcão, A., Lopes, J. A., & Silva, L. R. (2025). Phenolic acids from fruit by-products as therapeutic agents for metabolic syndrome: A review. *Int. J. Mol. Sci.*, *26*(8), 3834. https://doi.org/10.3390/ijms26083834

Rushdy, M. N. Q., Awang, M. A., Benjamin, M. A. Z., Saini, A., Mohd Rosdan, M. D. E., & Somboon, P. (2026). Extraction of *Gnetum gnemon* L. (melinjo) leaves using deep eutectic and conventional solvents: Phytochemical contents, antioxidant capacity, and toxicity assessment. *Trends Sci.*, *23*(2), 11392. https://doi.org/10.48048/tis.2026.11392

Saini, A., Benjamin, M. A. Z., Mohd Rosdan, M. D. E., Mohamad Rosdi, M. N., & Awang, M. A. (2025a). Phenolic and chlorogenic acid recovery from *Solanum lasiocarpum* Dunal (terung asam) via solid-phase extraction: Fractionation, antioxidant, molecular docking, and anti-obesity. *Malays. J. Anal. Sci.*, *29*(3), 1460.

Saini, A., Benjamin, M. A. Z., Mohd Rosdan, M. D. E., Mohamad Rosdi, M. N., & Awang, M. A. (2025b). Response surface methodology-based optimised ultrasound-assisted extraction of phenolics from *Solanum lasiocarpum* Dunal (terung asam) fruit: Anti-diabetic evaluation and ADMET study. *Biocatal. Agric. Biotechnol.*, *68*, 103704. https://doi.org/10.1016/j.bcab.2025.103704

Samary, K., Salehi, F., Aliverdi, A., & Daraei Garmakhany, A. (2025). Effect of magnetized water and magnetic field treatments on the physicochemical properties, total phenolic and antioxidant capacity of sprouted oats flour. *Innov. Food Technol.*, *12*(3), 273–285. https://doi.org/10.22104/IFT.2025.7738.2225

Shahidi, F., & Athiyappan, K. D. (2025). Polyphenol-polysaccharide interactions: Molecular mechanisms and potential applications in food systems – A comprehensive review. *Food Prod. Process. Nu.*, *7*(1), 42. https://doi.org/10.1186/s43014-025-00322-3

Shi, C., Guo, C., Wang, S., Li, W., Zhang, X., Lu, S., Ning, C., & Tan, C. (2024). The mechanism of pectin in improving anthocyanin stability and the application progress of their complexes: A review. *Food Chem.: X*, *24*, 101955. https://doi.org/10.1016/j.fochx.2024.101955

Soon, A. T. K., & Ding, P. (2021). A review on wild indigenous eggplant, terung asam Sarawak (*Solanum lasiocarpum* Dunal.). *Sains Malaysiana*, *50*(3), 595–603. https://doi.org/10.17576/jsm-2021-5003-03

Stachelska, M. A., Karpiński, P., & Kruszewski, B. (2025). A comprehensive review of biological properties of flavonoids and their role in the prevention of metabolic, cancer and neurodegenerative diseases. *Appl. Sci.*, *15*(19), 10840. https://doi.org/10.3390/app151910840

Tian, Y., Zhou, Y., Kriisa, M., Anderson, M., Laaksonen, O., Kütt, M.-L., Föste, M., Korzeniowska, M., & Yang, B. (2023). Effects of fermentation and enzymatic treatment on phenolic compounds and soluble proteins in oil press cakes of canola (*Brassica napus*). *Food Chem.*, *409*, 135339. https://doi.org/10.1016/j.foodchem.2022.135339

Veer, S. J., Pawase, P. A., Khalid, S., Dattatraya, R. H., Bashir, O., & Abdi, G. (2026). Pectinase-assisted extraction of red dragon fruit (*Hylocereus polyrhizus*) juice: Process optimization and quality assessment. *Food Chem. Adv.*, *10*, 101244. https://doi.org/10.1016/j.focha.2026.101244

Vidana Gamage, G. C., & Choo, W. S. (2023). Effect of hot water, ultrasound, microwave, and pectinase-assisted extraction of anthocyanins from black goji berry for food application. *Heliyon*, *9*(3), e14426. https://doi.org/10.1016/j.heliyon.2023.e14426

Villacís-Chiriboga, J., Elst, K., Van Camp, J., Vera, E., & Ruales, J. (2020). Valorization of byproducts from tropical fruits: Extraction methodologies, applications, environmental, and economic assessment: A review (Part 1: General overview of the byproducts, traditional biorefinery practices, and possible applications). *Compr. Rev. Food Sci. Food Saf.*, *19*(2), 405–447. https://doi.org/10.1111/1541-4337.12542

Vogelsang-O'Dwyer, M., Sahin, A. W., Arendt, E. K., & Zannini, E. (2022). Enzymatic hydrolysis of pulse proteins as a tool to improve techno-functional properties. *Foods*, *11*(9), 1307. https://doi.org/10.3390/foods11091307

Wen, L., Chen, J., Wang, J., & Yang, B. (2025). Carbohydrase-assisted extraction of phenolic compounds from citrus peel: Chemical composition and cellular antioxidant activity. *J. Sci. Food Agric.* https://doi.org/10.1002/jsfa.70267

World Health Organization. (2024). *Obesity and overweight*. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Wu, J., Ao, T., Yuan, Y., Wan, Z., Chandra, R., & Saddler, J. (2026). The key role that cellulose accessibility plays in restricting enzyme-mediated hydrolysis of cellulose. *Biotechnol. Adv.*, *87*, 108780. https://doi.org/10.1016/j.biotechadv.2025.108780