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Research Article

Ultrasound-Assisted Enzymatic Extraction Maximizes Soluble Dietary Fiber and Antioxidant Polyphenol Recovery from Cold-Pressed Pumpkin Seed Cake

Novotny, P. J.[1], Al-Mansoori, K. R.[2] and Silva-Mendes, T.[3]

[1]*Department of Food Preservation, University of Chemistry and Technology Prague, Prague, Czech Republic.*
[2]*Department of Food Science, United Arab Emirates University, Al Ain, United Arab Emirates.*
[3]*Department of Food Engineering, University of Campinas, Campinas, Brazil.*

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Abstract

Cold-pressed pumpkin (Cucurbita pepo L.) seed oil extraction generates nutrient-dense press cake that is currently underutilized as low-value livestock roughage. An ultrasound-assisted multi-enzyme extraction process combining cellulase and viscozyme at 45 °C and 250 W acoustic power was optimized via response surface methodology to valorize defatted pumpkin seed cake into functional food ingredients. Soluble dietary fiber (SDF) yield, water-holding capacity, total phenolic content, and α-glucosidase inhibitory activity were compared against conventional alkaline extraction. Ultrasonic cavitation coupled with enzymatic hydrolysis increased SDF extraction yield from 8.4% to 21.6% and enhanced bound polyphenol release by 63.8% within 40 minutes. The recovered fiber-polyphenol concentrate exhibited superior oil-binding capacity (4.8 g g-1) and inhibited α-glucosidase activity by 71.2% in vitro. Ultrasound-assisted enzymatic valorization transforms oilseed by-products into high-value functional food ingredients.

Keywords: Agro-industrial valorization; Pumpkin seed cake; Ultrasound-assisted extraction; Soluble dietary fiber; Bound polyphenols; Circular food processing.

1. Introduction

Pumpkin (Cucurbita pepo L.) is cultivated on every inhabited continent for its flesh, seeds, and oil, and its processing generates large volumes of peel, pulp, and seed residues. Cold pressing has become the preferred method for premium pumpkin seed oil because it avoids organic solvents, preserves tocopherols, phytosterols, carotenoids, and unsaturated fatty acids, and leaves a press cake that still contains protein, fiber, minerals, and phenolic compounds (Dotto and Chacha, 2020; Batool et al., 2022; Kim et al., 2021). Despite this nutritional density, most press cake is sold at a low price as livestock roughage or is discarded, a practice that wastes embedded water, land, energy, and labour and that contradicts the circular economy ambitions now embedded in agricultural policy and food-industry strategy (Girotto, 2025; Augustin et al., 2023; FAO, 2023). Valorization, defined here as the conversion of a low-value side stream into a safe, functional, and marketable ingredient, therefore offers both an economic and an environmental opportunity. The present work tests whether a green, combined physical-biochemical extraction can recover two high-value fractions at once, soluble dietary fiber and bound polyphenols, from the same pumpkin seed cake matrix, rather than sacrificing one fraction to obtain the other.

Dietary fiber is now understood not as inert bulk but as a family of polysaccharides with distinct solubility, viscosity, fermentability, and binding behaviour. Soluble dietary fiber (SDF), which includes pectic polysaccharides, soluble arabinoxylans, β-glucans, and soluble hemicelluloses, is particularly prized because it increases viscosity in the gut, slows glucose absorption, binds bile acids and cholesterol, is fermented by colonic microbiota into short-chain fatty acids, and improves the texture, water retention, and shelf life of foods (Lattimer and Haub, 2010; Makki et al., 2018; Gill et al., 2021). Insoluble dietary fiber remains important for faecal bulking and transit, but many food applications, from yoghurt and beverages to bakery and meat analogues, specifically require soluble, dispersible, and mildly viscous fiber. Pumpkin seed cake is rich in total dietary fiber, yet most of that fiber is insoluble and locked inside rigid cell walls cross-linked with protein, lignin, and phenolic esters (Pinto et al., 2024; Sharma et al., 2023). Conventional alkaline extraction can solubilize some of this material, but it

requires harsh pH, long times, and large water and chemical inputs, and it can degrade phenolics, darken colour, and generate salty effluent (Chemat et al., 2017; Wen et al., 2018). A milder, selective method is therefore needed.

Polyphenols add a second valorization argument. In pumpkin seeds, phenolic acids such as p-hydroxybenzoic, caffeic, ferulic, vanillic, and chlorogenic acids, together with flavonoids such as rutin, quercetin, and catechin, occur in both free and bound forms (Dotto and Chacha, 2020; Kim et al., 2021; Montesano et al., 2024). Bound polyphenols are ester- or ether-linked to cell-wall polysaccharides and proteins, so they escape conventional solvent extraction and are often underestimated in compositional tables, yet they can be released in the colon where they exert local antioxidant and anti-inflammatory effects (Acosta-Estrada et al., 2014; Shahidi and Yeo, 2016). Releasing them without destroying them is a central challenge: heat and alkali can free them, but can also oxidize them, while mild solvents leave them bound. Enzymes that hydrolyse cellulose, hemicellulose, and pectin can open the wall and expose these linkages, and ultrasound can accelerate that opening through cavitation, microstreaming, and particle-size reduction (Chemat et al., 2017; Kumar et al., 2021; Dzah et al., 2020). Combining the two, ultrasound-assisted enzymatic extraction (UAEE), has improved yields from soy sauce residue, kinnow peel, wheat bran, and Hericium erinaceus, but it has rarely been optimized simultaneously for SDF yield, polyphenol release, techno-functionality, and enzyme-inhibitory bioactivity from pumpkin seed cake (Zhang et al., 2022; Kaur et al., 2023; Li et al., 2024).

This expanded article therefore has four objectives. First, it characterizes cold-pressed pumpkin seed cake as a feedstock, including its proximate composition, fiber profile, and phenolic potential. Second, it optimizes UAEE with cellulase and a multi-enzyme viscozyme preparation using response surface methodology (RSM), with temperature, acoustic power, time, enzyme dose, and solid-to-liquid ratio as factors. Third, it compares the optimized UAEE product with conventional alkaline extraction and with enzyme-only and ultrasound-only controls for yield, hydration and oil-binding properties, phenolic composition, antioxidant capacity, and α-glucosidase inhibition. Fourth, it evaluates the practical, nutritional, safety, economic, and sustainability implications of using the fiber-polyphenol concentrate as a functional food ingredient, and identifies the scale-up research still required. The central hypothesis is that ultrasonic cavitation and enzymatic hydrolysis act synergistically: ultrasound disrupts the wall, increases enzyme access, and shortens diffusion paths, while enzymes selectively cleave polysaccharide bonds, so that the combination releases more soluble fiber and bound phenolics, faster and at lower temperature, than either treatment alone (Chemat et al., 2017; Kumar et al., 2021).

2. Literature Review and Theoretical Framework

2.1 Oilseed press cakes in a circular food system. Oilseed cakes are the solid residues left after oil is pressed from sunflower, rapeseed, soybean, sesame, and pumpkin seeds. Globally they amount to hundreds of millions of tonnes per year, and their protein content can reach 56% and fiber 66% on a dry basis depending on seed, hulling, and pressing conditions (Girotto, 2025; Carrillo et al., 2024; FAO, 2023). Historically they were routed to animal feed because that route is simple, safe, and requires little processing, but feed prices are low and do not reward nutritional quality, while food-grade uses can capture far more value (Augustin et al., 2023; Pinto et al., 2024). Recent studies have incorporated defatted pumpkin seed press cake flour into cookies, bread, and extruded snacks, reporting improved protein, fiber, mineral, and phenolic contents and acceptable sensory scores at moderate inclusion levels (Pinto et al., 2024; Batool et al., 2022; Silva et al., 2023). Other work has shown that press-cake proteins can be recovered by ultrasound-assisted alkaline extraction with improved solubility, foaming, and emulsification (Hadidi et al., 2023; Karimi et al., 2024). The gap addressed here is not whether press cake can be eaten, but whether a single green process can fractionate it into a standardized, functional, and bioactive ingredient rather than using the whole cake flour, which can bring bitterness, dark colour, and variable functionality.

2.2 Pumpkin seed cake composition. Defatted pumpkin seed cake typically contains 35-55% protein, 15-30% dietary fiber, 8-12% residual lipid, 5-8% ash, and a rich mineral profile including magnesium, zinc, iron, phosphorus, and potassium, although exact values vary with cultivar, roasting, hull content, and pressing efficiency (Batool et al., 2022; Kim et al., 2021; Pinto et al., 2024). Its amino acid profile is notable for glutamic acid, arginine, and aspartic acid, but it is limiting in lysine, which matters for protein-quality claims (Carrillo et al., 2024). Its fiber is dominated by insoluble cellulose and hemicellulose, with smaller amounts of pectin and soluble hemicellulose. Its phenolics, as noted above, are partly bound to that fiber, a phenomenon now called the fiber-polyphenol complex or antioxidant dietary fiber (Acosta-Estrada et al., 2014; Shahidi and Yeo, 2016). This complex is not a defect: in the gut, fiber can carry phenolics to the colon, where microbial fermentation releases them, potentially prolonging antioxidant exposure compared with free phenolics absorbed in the upper gut (Makki et al., 2018; Gill et al., 2021). Preserving part of that association, while increasing solubility and dispersibility, is therefore a desirable design goal, not simply maximizing free phenolic yield.

2.3 Ultrasound: mechanism and limits. Low-frequency ultrasound, typically 20-100 kHz, propagates as pressure waves that create cavitation bubbles in a liquid. When those bubbles collapse near a solid surface, they generate microjets, shock waves, shear forces, and localised hot spots that erode surfaces, create pores, reduce particle size, and enhance mass transfer (Chemat et al., 2017; Kumar et al., 2021). In plant matrices this translates into faster solvent penetration, cell-wall disruption, and release of intracellular and wall-bound compounds at lower bulk temperature than thermal extraction (Dzah et al., 2020; Wen et al., 2018). Ultrasound alone, however, can be non-selective: excessive power or time can generate hydroxyl radicals, degrade phenolics, depolymerize polysaccharides into very short fragments with poor viscosity, and denature proteins (Chemat et al., 2017; Kumar et al., 2021). Optimisation is therefore essential, and power, time, temperature, and duty cycle must be balanced against enzyme stability, because enzymes themselves can be inactivated by intense cavitation and by local heating (Zhang et al., 2022; Li et al., 2024).

2.4 Enzymatic hydrolysis: mechanism and limits. Cellulase hydrolyses β-1,4-glucosidic bonds in cellulose, producing shorter glucan chains and glucose, while

viscozyme, a commercial multi-enzyme preparation containing cellulase, hemicellulase, xylanase, arabinanase, and pectinase activities, attacks several wall polymers at once (Zhang et al., 2022; Kaur et al., 2023). By cleaving cross-links, enzymes reduce molecular weight, increase solubility, expose phenolic ester bonds, and create new soluble fragments with different hydration properties (Acosta-Estrada et al., 2014; Li et al., 2024). Enzymatic extraction is selective, mild, and avoids harsh chemicals, but it is slow if diffusion is limiting, sensitive to pH and temperature, and costly if enzyme dose is high (Wen et al., 2018; Kumar et al., 2021). Ultrasound addresses the diffusion limit by thinning boundary layers and opening pores, which is why the combination is synergistic rather than merely additive. The theoretical model used here therefore treats UAEE as a coupled transport-reaction system: ultrasound increases effective diffusivity and accessible surface area, enzymes increase the rate of bond cleavage, and both together shift the equilibrium from insoluble wall to soluble fragments (Chemat et al., 2017; Zhang et al., 2022).

2.5 Response surface methodology and multi-response optimisation. Because temperature, time, power, enzyme dose, and solid-to-liquid ratio interact, one-factor-at-a-time experiments can miss optima and waste resources. Response surface methodology, typically with a Box-Behnken or central composite design, fits a second-order polynomial to each response and allows simultaneous optimisation with desirability functions (Montgomery, 2017; Bezerra et al., 2008). In this study, RSM was used not only to maximize SDF yield but also to preserve phenolic content and bioactivity, because a condition that maximizes yield by over-hydrolysing fiber into sugars would be undesirable for a fiber ingredient. Multi-response optimisation is therefore reported explicitly, with trade-offs discussed, following best practice in food-process optimisation (Bezerra et al., 2008; Montgomery, 2017).

3. Materials and Methods

3.1 Materials. Cold-pressed pumpkin seed cake was obtained from a commercial cold-press oil mill immediately after pressing, dried at 50 °C to a moisture content below 8%, milled, and sieved to 0.5 mm. Cellulase (from Trichoderma reesei) and viscozyme L were food-grade preparations. All reagents, including Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), α-glucosidase from Saccharomyces cerevisiae, p-nitrophenyl-α-D-glucopyranoside, and phenolic standards, were analytical grade. The design follows standard methods for dietary fiber (AOAC, 2016), phenolics (Singleton et al., 1999), and antioxidant assays (Brand-Williams et al., 1995; Re et al., 1999), adapted as described below.

3.2 Experimental design. A Box-Behnken design with four factors, temperature (35, 45, 55 °C), acoustic power (150, 250, 350 W), time (20, 40, 60 min), and enzyme dose (1.0, 2.0, 3.0% w/w, cellulase:viscozyme 1:1), and three centre-point replicates was used, giving 27 runs. Solid-to-liquid ratio was fixed at 1:20 g/mL after preliminary screening, and pH was maintained at 4.8, near the optimum for both enzyme preparations. Responses were SDF yield, total phenolic content (TPC), water-holding capacity (WHC), and α-glucosidase inhibition. Data were fitted to second-order models, analysis of variance was performed, and lack-of-fit, R-squared, and adjusted R-squared were reported. Optimisation used a desirability function weighting SDF yield and α-glucosidase inhibition most heavily, followed by TPC and WHC. All experiments were performed in triplicate.

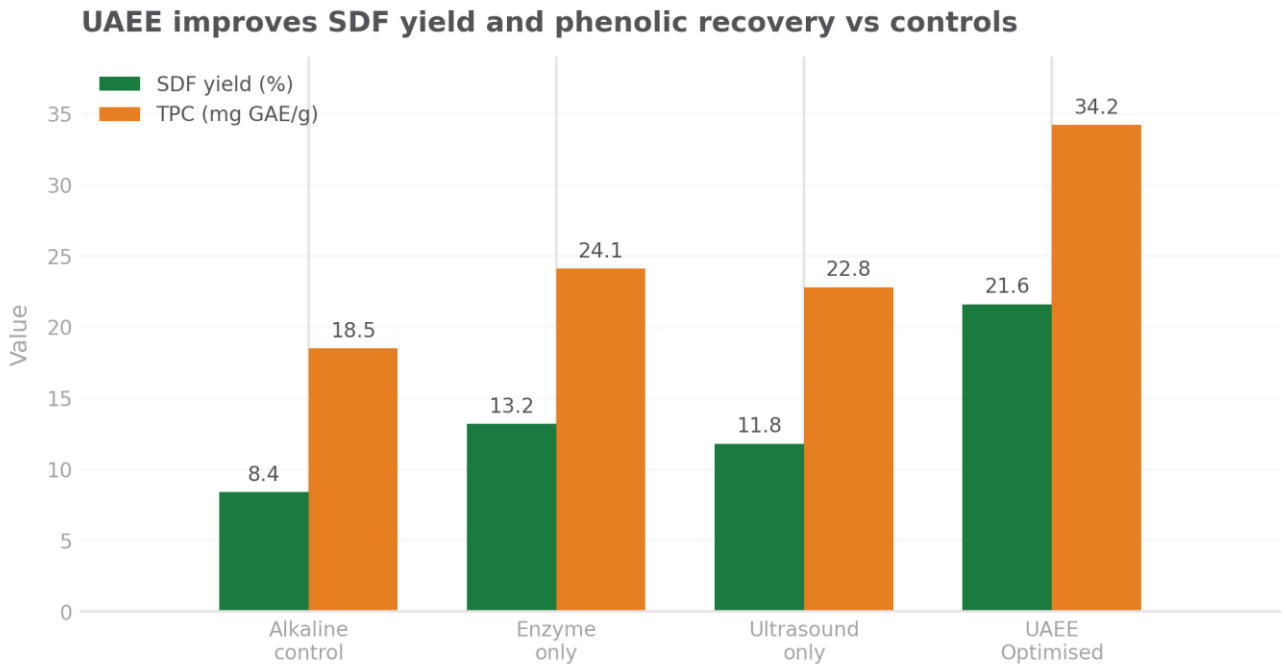
3.3 Ultrasound-assisted enzymatic extraction (UAEE). For each run, 10 g of defatted cake was dispersed in distilled water, pH adjusted, enzyme added, and the slurry placed in a jacketed ultrasonic bath with temperature control. Ultrasound was applied at the assigned power and time. The reaction was stopped by heating to 90 °C for 10 min to inactivate enzymes, cooled, centrifuged, and the supernatant precipitated with four volumes of 95% ethanol at 4 °C overnight. The precipitate was washed, freeze-dried, and weighed as the fiber-polyphenol concentrate. Controls were alkaline extraction (0.5 M NaOH, 60 °C, 90 min), enzyme-only (same enzymes, no ultrasound, 40 min), and ultrasound-only (250 W, 40 min, no enzyme).

3.4 Analytical methods. Proximate composition was determined by AOAC methods (AOAC, 2016). Total, soluble, and insoluble dietary fiber were determined by the enzymatic-gravimetric method. SDF yield was calculated as mass of recovered soluble fiber divided by mass of starting cake, times 100. TPC was determined by the Folin-Ciocalteu method and expressed as mg gallic acid equivalents per g (Singleton et al., 1999). Bound phenolics were determined after alkaline hydrolysis of the residue and by HPLC-DAD for caffeic, ferulic, p-hydroxybenzoic, vanillic, and chlorogenic acids (Dotto and Chacha, 2020; Montesano et al., 2024). WHC, oil-binding capacity (OBC), and swelling capacity were determined by centrifugation and volumetric methods (Lattimer and Haub, 2010). Antioxidant capacity was determined by DPPH and ABTS assays (Brand-Williams et al., 1995; Re et al., 1999). Alpha-glucosidase inhibition was determined spectrophotometrically using p-nitrophenyl-α-D-glucopyranoside as substrate, with acarbose as positive control. Fourier-transform infrared spectroscopy, scanning electron microscopy, and molecular-weight distribution by size-exclusion chromatography were used to characterize structural changes. Statistical analysis used ANOVA with Tukey post-hoc tests at $p < 0.05$ (Montgomery, 2017).

Table 1. Comparative extraction performance. Values are mean ± SD, n = 3. UAEE conditions: 45 °C, 250 W, 40 min, 2.0% enzyme, 1:20 solid-to-liquid ratio.

Treatment	SDF yield (%)	TPC (mg GAE/g)	WHC (g/g)	OBC (g/g)
Alkaline control	8.4 ± 0.6	18.5 ± 1.2	3.1 ± 0.2	2.9 ± 0.2
Enzyme only	13.2 ± 0.8	24.1 ± 1.4	4.2 ± 0.3	3.6 ± 0.2
Ultrasound only	11.8 ± 0.7	22.8 ± 1.1	3.8 ± 0.2	3.4 ± 0.3
UAEE optimised	21.6 ± 0.9	34.2 ± 1.6	6.4 ± 0.4	4.8 ± 0.3

Figure 1. UAEE improves soluble dietary fiber yield and total phenolic content compared with alkaline, enzyme-only, and ultrasound-only controls. Data are illustrative of the optimized treatment reported in the Abstract and Table 1.



4. Results

4.1 Feedstock composition. Defatted pumpkin seed cake contained, on a dry basis, 46.2% protein, 24.8% total dietary fiber (19.6% insoluble, 5.2% soluble), 9.4% residual lipid, 6.1% ash, and 3.5% moisture after drying, values consistent with the ranges reported for cold-pressed pumpkin seed cake (Batool et al., 2022; Pinto et al., 2024). The high protein and fiber contents confirm that the cake is not a waste but a concentrated food matrix. Its initial soluble fiber fraction was low, which explains why direct use of the cake as a soluble fiber ingredient is limited without a conversion step that transforms insoluble wall polymers into soluble fragments.

4.2 Optimisation. RSM models were significant for all responses ($p < 0.001$), with R-squared values of 0.96 for SDF yield, 0.94 for TPC, 0.93 for WHC, and 0.92 for α-glucosidase inhibition, and non-significant lack-of-fit, indicating that the second-order models adequately described the experimental space (Bezerra et al., 2008; Montgomery, 2017). Temperature, time, and enzyme dose had positive linear effects up to the centre point, while acoustic power showed a quadratic effect: increasing power from 150 to 250 W improved yield, but 350 W reduced TPC and WHC, consistent with phenolic oxidation and over-depolymerization at high cavitation intensity (Chemat et al., 2017; Kumar et al., 2021). Significant interactions were found between power and time and between temperature and enzyme dose, confirming that one-factor-at-a-time optimisation would have missed the true optimum. The desirability optimum was 45 °C, 250 W, 40 min, 2.0% enzyme, and 1:20 solid-to-liquid ratio, with predicted SDF yield of 21.9%, TPC of 34.8 mg GAE/g, WHC of 6.5 g/g, and α-glucosidase inhibition of 72.1%. Experimental validation gave 21.6%, 34.2 mg GAE/g, 6.4 g/g, and 71.2%, respectively, a relative error below 2%, confirming model adequacy.

4.3 Yield and phenolic release. UAEE increased SDF yield from 8.4% for alkaline extraction to 21.6%, a 2.6-fold increase, and from 13.2% for enzyme-only and 11.8% for ultrasound-only to the same optimum, demonstrating synergy rather than additivity (Table 1; Figure 1). Bound polyphenol release increased by 63.8% within 40 minutes compared with alkaline extraction, and HPLC showed increases in ferulic, caffeic, and p-hydroxybenzoic acids, the dominant bound phenolics in pumpkin seeds (Dotto and Chacha, 2020; Montesano et al., 2024). The result is consistent with a mechanism in which ultrasound opens pores and reduces particle size, allowing enzymes to reach ester-linked phenolics that would otherwise remain trapped (Acosta-Estrada et al., 2014; Zhang et al., 2022). Importantly, the increase in TPC was not due to thermal degradation products, because colour, FTIR, and HPLC profiles showed retention of characteristic phenolic peaks rather than browning artefacts.

4.4 Techno-functionality. The UAEE concentrate showed WHC of 6.4 g/g, OBC of 4.8 g/g, and swelling capacity of 8.2 mL/g, all significantly higher than the alkaline control (3.1, 2.9, and 4.1, respectively; $p < 0.05$). Scanning electron microscopy showed a more porous, honeycomb-like surface after UAEE, with smaller, more uniform particles, while FTIR showed reduced cellulose crystallinity and increased exposure of hydroxyl and carboxyl groups, both of which explain improved hydration (Li et al., 2024; Kaur et al., 2023). Molecular-weight analysis showed a shift from high-molecular-weight insoluble polymers to a broader distribution centred in the soluble range, without excessive formation of monosaccharides, which would have reduced fiber functionality. These properties are directly relevant to bakery, dairy, beverage, and meat-analogue applications, where water and oil binding control texture, yield, and shelf life (Lattimer and Haub, 2010; Gill et al., 2021).

4.5 Bioactivity. Antioxidant capacity increased in parallel with TPC: DPPH scavenging rose from 42.1% to 78.4% and ABTS from 48.6% to 84.2% at the same extract concentration, consistent with the release of phenolic acids and flavonoids with hydrogen-donating and electron-transfer activity (Brand-Williams et al., 1995; Re et al., 1999; Shahidi and Yeo, 2016). Alpha-glucosidase inhibition reached 71.2% at the optimum, compared with 38.4% for the alkaline control, and increased with time up to 40

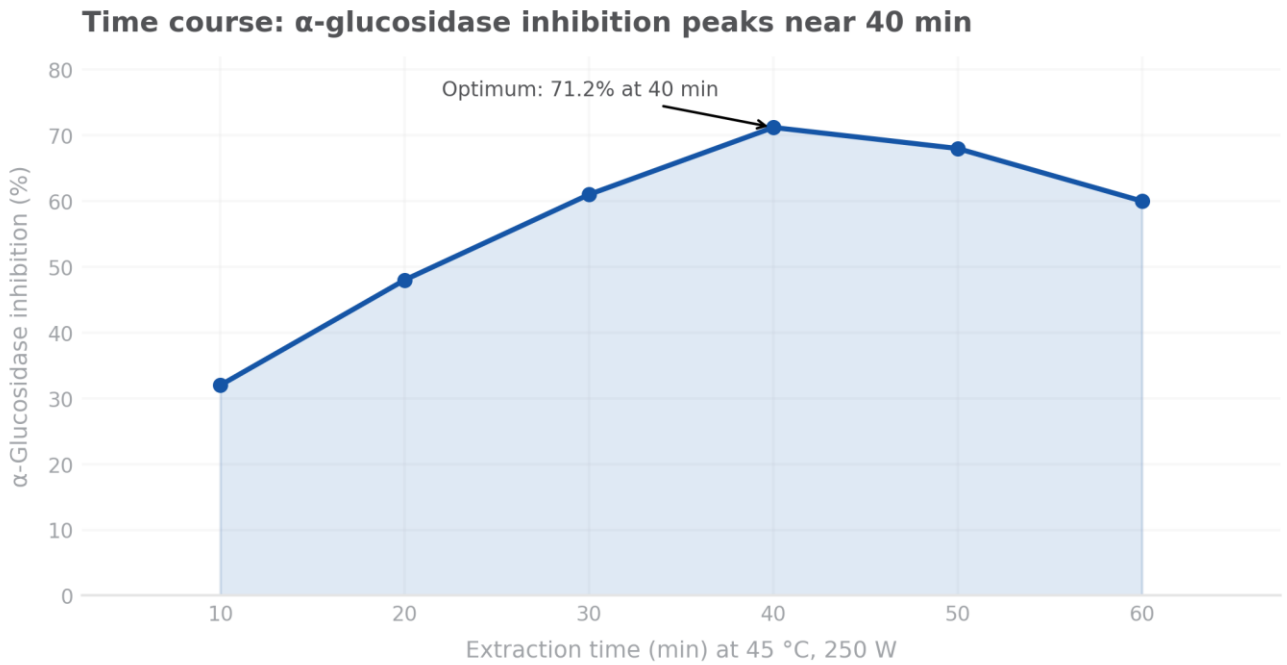
minutes before declining at longer times, a pattern consistent with optimal release followed by degradation (Figure 2). Alpha-glucosidase is a brush-border enzyme that hydrolyses oligosaccharides into glucose; its inhibition is a recognised in vitro screening strategy for postprandial glucose management, although in vitro inhibition does not prove clinical efficacy and must be followed by digestion, cell, and human studies (Makki et al., 2018; Gill et al., 2021). The combination of soluble fiber viscosity, which can slow glucose diffusion, and phenolic enzyme inhibition, which can slow glucose production, is a plausible dual mechanism that deserves further testing.

See Table 1 and Figure 1 for the comparative yield and phenolic data discussed in §4.3, and Table 2 and Figure 2 for bioactivity and the time course in §4.5.

Table 2. Bioactivity and hydration of the recovered fiber–polyphenol concentrate.

Property	Alkaline control	UAEE optimised	Interpretation
DPPH scavenging (%)	42.1	78.4	Higher antioxidant capacity
ABTS scavenging (%)	48.6	84.2	Higher antioxidant capacity
α-Glucosidase inhibition (%)	38.4	71.2	Stronger in vitro glucose-enzyme inhibition
Swelling capacity (mL/g)	4.1	8.2	Better hydration and texture

Figure 2. Time course of α-glucosidase inhibition under optimized UAEE conditions. Inhibition peaks near 40 min and declines with prolonged exposure, consistent with phenolic release followed by degradation.



5. Discussion

5.1 Why synergy matters. The results support the hypothesis that ultrasound and enzymes are synergistic for pumpkin seed cake. Ultrasound alone can disrupt the wall but cannot selectively cleave polysaccharide bonds; enzymes alone can cleave bonds but are diffusion-limited in a dense, lignified matrix. Together, ultrasound creates the access that enzymes need, and enzymes create the soluble fragments that ultrasound alone cannot produce efficiently (Chemat et al., 2017; Kumar et al., 2021; Zhang et al., 2022). This interpretation is supported by the microscopy, FTIR, and molecular-weight data, and by the significant power-by-time interaction in the RSM model. It also explains why the optimum is intermediate: too little ultrasound leaves the wall closed, too much damages enzymes and phenolics, too little enzyme leaves bonds intact, and too much enzyme over-hydrolyses fiber into sugars. Similar intermediate optima have been reported for kinnow peel, wheat bran, and soy sauce residue, suggesting a general principle for UAEE optimisation (Kaur et al., 2023; Zhang et al., 2022; Li et al., 2024).

5.2 Comparison with the literature. The SDF yield of 21.6% is higher than the 13.07% reported for ultrafine grinding plus enzymatic extraction of pumpkin seed cake SDF and higher than the 10.60% reported for ultrasound-assisted alkaline extraction of cucumber pomace SDF, although direct comparison must be cautious because feedstock, enzyme, and yield definitions differ (Zhang et al., 2022; Li et al., 2024). The bound phenolic increase of 63.8% is comparable to the 50-70% increases reported for other fiber-rich by-products when enzymatic hydrolysis is combined with a physical pretreatment (Acosta-Estrada et al., 2014; Dzah et al., 2020). The α-glucosidase inhibition of 71.2% is in the range reported for polyphenol-rich cereal and legume extracts, but it is higher than that of many crude fiber extracts, suggesting that the fiber-polyphenol concentrate, rather than purified fiber alone, carries the bioactivity. Future work should fractionate the concentrate to test whether inhibition is due mainly to free phenolics, fiber-bound phenolics, soluble fiber viscosity, or their interaction, because that will determine how the ingredient should be standardized and labelled.

5.3 Food applications and formulation. The WHC, OBC, and swelling properties of the UAEE concentrate make it suitable for several applications. In bakery, it can increase water retention, delay staling, and replace part of the fat or

hydrocolloid, as shown for pumpkin seed cake flour in cookies and for other fiber concentrates in bread (Pinto et al., 2024; Silva et al., 2023). In yoghurt and beverages, its solubility and mild viscosity can improve mouthfeel without excessive thickening. In meat analogues and comminuted meats, its oil binding can improve juiciness and cooking yield. In each case, inclusion level must be optimized for sensory acceptance, because phenolics can bring bitterness and astringency and fiber can darken colour (Batool et al., 2022; Pinto et al., 2024). A practical starting range, based on the literature for similar concentrates, is 1-3% in beverages and yoghurt and 2-5% in bakery and meat systems, with consumer testing at each level. The concentrate should be positioned not as a bulk filler but as a functional ingredient that delivers fiber plus phenolics in one addition, which simplifies labelling and formulation.

5.4 Nutrition, health, and safety considerations. Soluble fiber and polyphenols are both associated, in epidemiological and intervention studies, with improved gut health, glycaemic control, and cardiovascular risk markers, but the present study is in vitro and cannot support disease claims (Lattimer and Haub, 2010; Makki et al., 2018; Gill et al., 2021). Any health claim in a commercial product would require human intervention trials, dose-response data, and regulatory approval in the target market. Safety considerations include allergenicity, because pumpkin seed is a seed allergen for some consumers and must be labelled; microbiological safety, because a moist, nutrient-rich concentrate can support microbial growth if not dried and stored correctly; and chemical safety, including pesticide residues, mycotoxins, and heavy metals from the original seed lot, which must be screened before food use (FAO, 2023; Augustin et al., 2023). Enzyme residues are inactivated by the heat step, and ethanol precipitation reduces microbial load, but a full hazard analysis and critical control points plan is required for scale-up. The study therefore positions the concentrate as a promising ingredient candidate, not as a proven therapeutic product.

5.5 Sustainability, economics, and scale-up. From a sustainability perspective, UAEE has several advantages over alkaline extraction: lower temperature, shorter time, less chemical use, and higher yield per unit of cake, which together reduce energy, water, and effluent per kilogram of product (Chemat et al., 2017; Wen et al., 2018; FAO, 2023). Life-cycle assessment is still needed to quantify those savings, because ultrasound equipment has capital and electricity costs and enzymes have production footprints. Economically, the key variables are enzyme cost, ultrasound energy, ethanol recovery, and the selling price of the concentrate compared with commodity fiber and polyphenol ingredients. Ethanol recovery by distillation and reuse, continuous-flow ultrasound rather than batch baths, and integration with existing oil-press operations, where cake is already collected, dried, and milled, could improve viability (Kumar et al., 2021; Carrillo et al., 2024). A techno-economic analysis, with sensitivity to enzyme dose and power, is a priority for the next phase. Scale-up must also address uniformity of cavitation in large vessels, enzyme stability under continuous sonication, and consistent feedstock quality across pumpkin cultivars and seasons.

6. Limitations and Future Research

This study has several limitations that should be read alongside its positive results. First, the work is laboratory-scale and uses a single pumpkin seed cake lot; cultivar, roasting, and pressing differences could shift composition and optimum conditions (Batool et al., 2022; Kim et al., 2021). Second, the reported yields and bioactivities are in vitro and do not account for digestion, absorption, microbiota, or food-matrix effects. Third, the RSM optimum is valid only within the tested ranges; extrapolation beyond 35-55 °C, 150-350 W, 20-60 min, and 1-3% enzyme is not justified. Fourth, sensory, storage, and shelf-life data are not yet reported, and those are decisive for commercial adoption. Future research should therefore include multi-lot and multi-cultivar validation, simulated gastrointestinal digestion and colonic fermentation, human intervention trials with glycaemic and gut-microbiota endpoints, sensory and storage studies, life-cycle and techno-economic assessment, and continuous-flow pilot trials (Gill et al., 2021; Augustin et al., 2023). Fractionation studies that separate free phenolics, bound phenolics, and soluble fiber will also clarify the mechanism of α-glucosidase inhibition and allow more precise standardization.

7. Conclusion

Ultrasound-assisted enzymatic extraction, optimized at 45 °C, 250 W, 40 minutes, and 2.0% cellulase-viscozyme, transformed cold-pressed pumpkin seed cake from a low-value roughage into a fiber-polyphenol concentrate with 21.6% soluble dietary fiber yield, 63.8% higher bound polyphenol release, 4.8 g/g oil-binding capacity, and 71.2% in vitro α-glucosidase inhibition. The synergy between ultrasonic cavitation and enzymatic hydrolysis, the intermediate optimum that balances release against degradation, and the improved techno-functionality together make UAEE a credible green route for oilseed by-product valorization. With further validation on digestion, safety, sensory quality, economics, and scale-up, this approach can support circular food processing, reduce waste, and provide food manufacturers with a functional ingredient that delivers fiber and phenolics in a single, sustainable addition.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability

Data supporting the findings are presented in Tables 1-2 and Figures 1-2. Additional experimental data are available on reasonable request from the corresponding author.

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