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RESEARCH ARTICLE

Combined effects of gamma irradiation and solvent-driven extraction on saponin and functional properties of okara

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Abstract

Soy saponins are amphiphilic triterpenoid glycosides valued for their surface activity and wide-ranging functionality and bioactivity. Okara, a by-product of soymilk production, is a potential source of saponins, but its recovery is limited by strong interactions with theinsoluble matrix. This study investigated the effects of gamma irradiation (GI- 0.5 kGy) applied as a seed-level pretreatment, combined with 70 % aqueous organic solvent (methanol, ethanol and acetone) extraction of varying polarity on total saponin recovery from okara. The purified extracts were then analysed for total saponin content (TSC) and evaluated for emulsifying capacity (EC) and foaming capacity (FC) as indicators of surface-active functionality. The results showed that irradiation significantly increased total saponin recovery across all solvent, with the highest increase observed with methanol (18.17 mg/g DW), representing a ~27 % rise compared with the corresponding non-irradiated control. Irradiated extracts also exhibit enhanced emulsifying (1.31–1.35-fold) and foaming capacities (1.39–1.42-fold). Multivariate analysis revealed strong positive correlations between saponin content and functional properties and clearly distinguished irradiated from non-irradiated samples, with methanol showing the strongest combined effect with irradiation. To the best of our knowledge, this study provides the first evidence that GI combined with polarity-optimised solvent extraction substantially enhances saponin yield and surface-active functionality in okara. Overall, these findings highlight okara as a value-added sustainable source of saponins with potential applications as natural surfactants and phytonutrients in food-related applications.

Keywords: functional properties; gamma irradiation; okara; solvent polarity; soy saponins; valorisation

Introduction

Okara, an insoluble by-product generated during soymilk and tofu production, is produced in large quantities worldwide and remains substantially underutilised despite its high content of proteins, dietary fiber and phytonutrients. Improper disposal of okara contributes to environmental pollution and economic losses, accentuating the pressing need for sustainable valorisation (1). In recent years, the recovery of high-value phytonutrients from agro-industrial by-product has gained increasing attention. Among the bioactive compounds present in okara, soy saponins have attracted particular interest due to their amphiphilic nature and multifunctional properties. Structurally, soy saponins are a class of intricate oleanane-type triterpenoid glycosides composed of a hydrophobic aglycone linked to hydrophilic sugar moieties, conferring excellent emulsifying, foaming and wetting characteristics. In addition to their technological functionality, soy saponins exhibit diverse biological activities, including antioxidant, anti-inflammatory, anticancer and anti-obesity effects, highlighting their potential applications in functional foods, nutraceuticals and pharmaceutical formulations (2, 3). However, the efficient extraction of saponins (0.1 g/100 g dry basis) from okara remains challenging, as these compounds are often embedded within a compact matrix of non-extractable cell-wall conjugates (4). Conventional extraction approaches rely primarily on organic solvents, with extraction efficiency strongly influenced by solvent polarity and affinity toward saponin aglycone-sugar complexes. The use of multiple organic solvents with differing polarities has therefore been recognised as an effective strategy to improve saponin recovery and selectivity. Nevertheless, solvent extraction alone may be insufficient to disrupt the tightly bound okara matrix, limiting mass transfer and phytonutrient release (5).

Gamma irradiation (GI) has become a promising non-thermal, residue-free technology that can cause controlled changes in the structure of plant-based matrices. At appropriate doses, GI weaken hydrogen bond and promote partial depolymerisation of complex macromolecule, thereby enhancing the accessibility and extractability of bound phytochemicals (6). When combined with solvent-assisted extraction, GI may act synergistically to improve saponin yield by facilitating solvent penetration and mass transfer. Despite growing interest in irradiation-assisted phytonutrient recovery, limited information is available regarding the combined effects of GI and solvent polarity

on the enhancement of saponin phytonutrients in okara. Therefore, the present study aims to evaluate the impact of GI in conjunction with three organic solvents (70 % of ethanol, methanol and acetone) of varying polarity on the extraction efficiency of total saponins from okara. The objectives were (i) to evaluate the effect of irradiation on total saponin extraction across different solvents and (ii) to examine how this treatment influences interfacial properties, including emulsifying (EC) and foaming capacities (FC). This work introduces GI combine with solvent based polarity optimised as a new processing approach for enhancing saponin recovery and functionality from okara. This integrated approach seeks to establish a sustainable valorisation route for soybean by-product, promoting the development of high -value functional ingredients.

Materials and Methods

Materials

The solvents acetone, methanol, ethanol, ethyl acetate, *n*-butanol and hexane and chemicals including vanillin and sulphuric acid were purchased from SRL (Bayswater, VIC, Australia), soy saponin I was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Soybean seeds of the DS9422 genotype (low kunitz trypsin inhibitor or LKTI) were procured from the Division of Genetics and Plant Breeding, ICAR– Indian Agricultural Research Institute (IARI), India and used as the initial material for the study. Seeds were cleaned to remove surface impurities, rinsed with distilled water, air-dried, packed in polyethylene zipper pouches and stored at 4 °C until further processing.

Pretreatment by gamma irradiation (GI)

Seed-level GI was carried out in a prior experiment using a ^{60}Co irradiation chamber (model GC-5000, BRIT, Mumbai) at ambient temperature (24 °C) at the Nuclear Research Laboratory (NRL), ICAR-IARI, India. The packed seed samples were irradiated at a dose of 0.5 kGy, while non-irradiated seeds served as a control. The irradiation dose was selected based on prior dose-response optimisation and reported effects on phytochemical composition (7). All subsequent analyses were performed in triplicate.

Sample preparation: production of soymilk by-product (okara)

Soymilk was prepared from the DS9422 yellow-seeded soybean genotype (LKTI) using both irradiated and non-irradiated seeds (300 g) according to the previously described methods (8). The seeds were soaked at 24 °C for 12 hr, rinsed and blended with distilled water at a 1:10 (w/v) ratio, followed by heating at 98 °C for 5 min. The slurry was filtered through muslin cloth to obtain soymilk. The freshly separated okara was dried at 45 °C for 14–16 hr, milled and sieved through an 80 µm mesh to produce uniform okara flour, which was stored at 4 °C until further analysis. The okara flour was defatted using *n*-hexane (1:10, w/v) under continuous stirring for 1 hr; the solvent was removed by vacuum filtration and the residue was air dried.

Comparison of extraction conditions

To evaluate the effects of solvent system and GI on saponin extraction, three extraction conditions were employed using aqueous organic solvents with solvent-compatible extraction process. Ethanol- and methanol-based extracts were subjected to *n*-butanol partitioning to selectively concentrate amphiphilic saponin fractions, whereas acetone extracts were processed via cold precipitation to recover saponins based on differential solubility (Fig. 1) (9).

Dried okara (1 g) was extracted with 20 mL of 70 % (v/v) organic solvent (methanol, ethanol and acetone) under continuous agitation (160 rpm) for 2 hr at 24 °C in the dark. The extract was filtered (Whatman no. 40) and concentrated by evaporation, reconstituted in 20 mL distilled water and transferred to a separatory funnel. An equal volume of *n*-butanol was added and the mixture was allowed to separate. The upper butanolic phase was collected and the partitioning was repeated once. The combined butanolic fractions were concentrated under reduced pressure, filtered (Whatman no. 1), dried to a powder, weighed and stored at 4 °C. For acetone extracts, *n*-butanol partitioning was omitted; instead, the aqueous residue was chilled at 4 °C for 2 hr to precipitate saponins (> 70 % purity), followed by centrifugation at 4500 × g for 10 min at 4 °C using a refrigerated centrifuge (model no. 5810R eppendrof, AG, Hamburg, Germany) and drying (9–11).

Determination of total saponin content (TSC)

Total saponin content in samples was quantified using a colorimetric vanillin-sulfuric acid assay with slight modification from established protocols. Briefly, 0.2 mL of each extract was reacted with 0.2 mL of 8 % (w/v) vanillin solution, followed by the addition of 2 mL of sulfuric acid (72 %, v/v) under ice-cold conditions to ensure controlled reaction initiation. The reaction mixture was subsequently incubated in a thermostatically controlled water bath at 60 °C for 15 min to allow chromophore development and then rapidly cooled in an ice bath for 10 min to terminate the reaction. The absorbance was measured at 544 nm. Total saponin content was then calculated from a standard curve constructed with purified soy saponin I standard (mg/g) (9–11).

Determination of emulsifying (EC) and foaming capacities (FC)

Emulsifying capacity was determined by preparing soybean oil–water emulsions (50:50, w/w) using 3.5 % of the extract as an emulsifier. The extract was dispersed in water, followed by the gradual addition of oil under continuous stirring (2000 rpm for 3 min) using digital magnetic stirrer (model no. BT-MSH-17D, BenchTop Lab Systems, USA). The emulsion was then centrifuged at 5000 × g for 10 min and EC was calculated as the ratio of the emulsified layer height to the total emulsion height was evaluated using a 2 % aqueous solution of the extract. The solution was vortexed for 10 min and FC was calculated from the increase in sample height after foaming (12).

Determination of explorative data analysis (EDA)

Explorative data analysis was performed in Python (version 3.10) using Google Colaboratory (Google LLC, Mountain View, CA, USA). The libraries pandas (v2.0), NumPy (v1.24), matplotlib (v3.7), seaborn (v0.12), NetworkX (v3.1) and scikit-learn (v1.3) were used to generate replicate dot strip plots, boxplots, interaction line plots, a Pearson correlation heatmap, a correlation network graph, a principal component analysis (PCA) biplot and a radar chart for TSC, EC and FC. Data for PCA were autoscaled prior to analysis using StandardScaler (13–19).

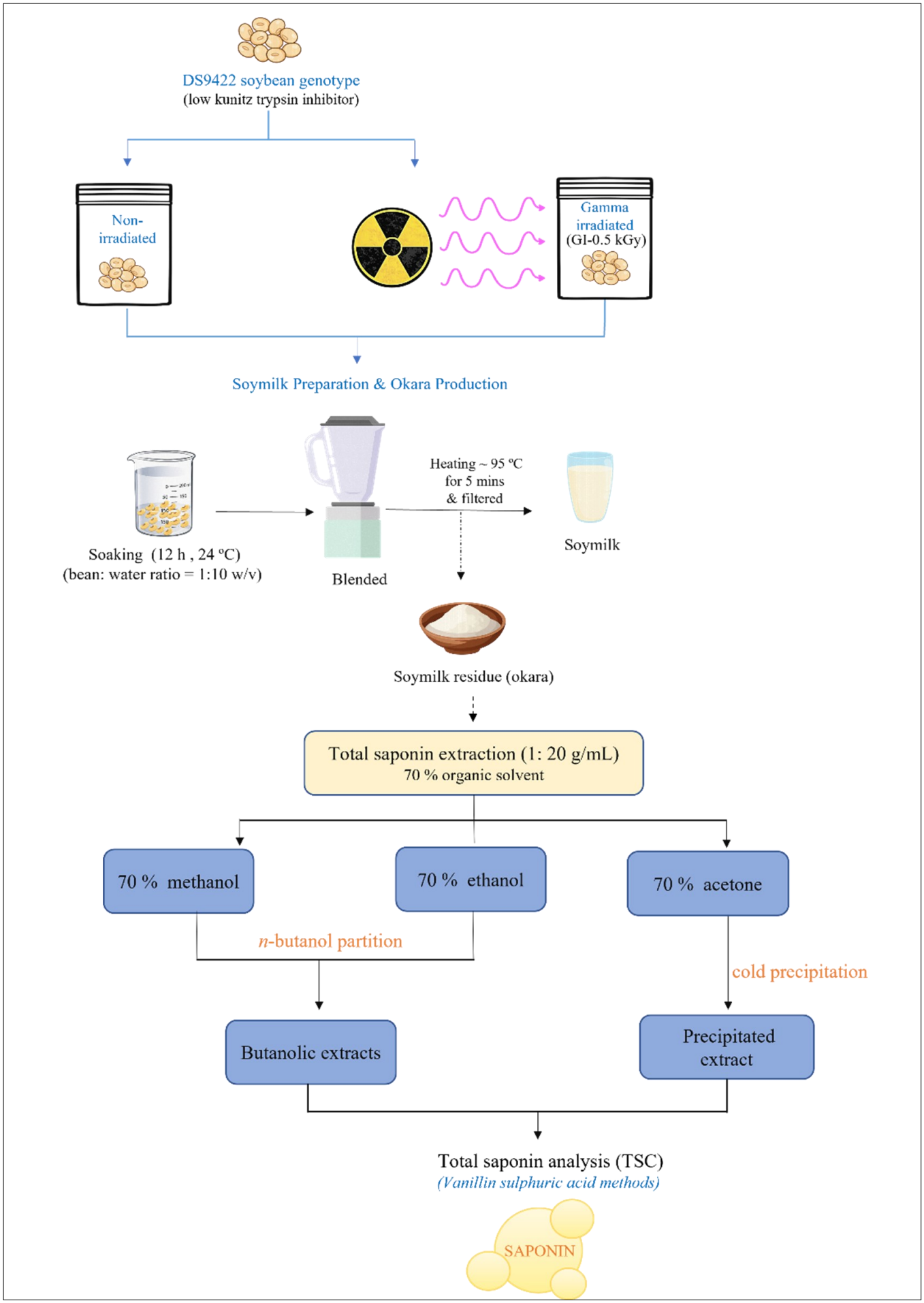

Fig. 1. Experimental workflow for soy okara saponin extraction and estimation.

Statistical analyses

All experiments were performed in triplicate and the data are expressed as mean ± standard deviation. Two-way analysis of variance (ANOVA) followed by Tukey's post hoc test was conducted at a significance level of $p < 0.05$ using GraphPad Prism version 10.0.0 (GraphPad Software, USA).

Results and Discussion

Effect of GI and solvent type on extraction of saponin

Gamma irradiation significantly enhanced the TSC across all extraction solvents ($p < 0.05$). Although GI treatment consistently produced higher TSC levels than non-irradiated samples, the effect's strength varied depending on the solvent. The greatest increase was observed in methanolic extracts, where TSC rise from 14.30 ± 0.56 to 18.17 ± 0.86 mg/g DW, followed by ethanol (12.70 ± 0.66 to 15.83 ± 0.91 mg/g DW) and acetone (10.97 ± 0.60 to 13.60 ± 0.20 mg/g DW). Despite these differences in magnitude, the treatment × solvent interaction was not significant ($p = 0.311$). These show that GI consistently influences TSC across all solvents (Fig. 2 A).

This study demonstrates that GI consistently enhances saponin recovery from okara across all solvent, indicating that irradiation functions as a primary factor independent of solvent systems. The non-significant irradiation × solvent interaction further suggests that the effect of irradiation is additive rather than solvent specific. While earlier studies reported that applying moderate to high irradiation doses (0.5–10 kGy) for antinutrient elimination have shown a dose dependent decline in saponin content (47–77 %) in quinoa flour, attributed to irradiation-induced cleavage of glycosidic bonds between the sugar moiety and steroidal or triterpenoid aglycones. In contrast, the present study employs a low GI dose (0.5 kGy) to enhance saponin extractability and functional recovery from okara rather than to eliminate saponins (7, 20). At this dose, GI of soybean seeds forms radicals from bound water that disrupt polysaccharide, initiating chain scission in β-1,4 glycosidic bonds of cellulose/hemicellulose, with structural effects retained in the okara matrix (21). In parallel, irradiation has been reported to enhance saponin biosynthesis by increasing the activity of key enzymes such as squalene synthase and oxido-squalene cyclase, thereby contributing to higher TSC (22).

Furthermore, GI disengages the compact okara ultrastructure attributed to enhance solvent penetration and mass transfer during extraction, thereby setting the stage for the total saponin recovery enhancement. The deeper ingress of solvent further influenced by solvent polarity, with methanol (dielectric constant, ε ≈ 33) providing more effective solvation of the polar sugar moieties of saponins than ethanol (ε ≈ 24.6) and acetone (ε ≈ 20.7). Saponin yield was highest in the n-butanol fraction, indicating preferential partitioning of amphiphilic saponins into this moderately polar solvent. This selectivity reduced co-extraction of polar impurities, resulting in fractions of > 70 %

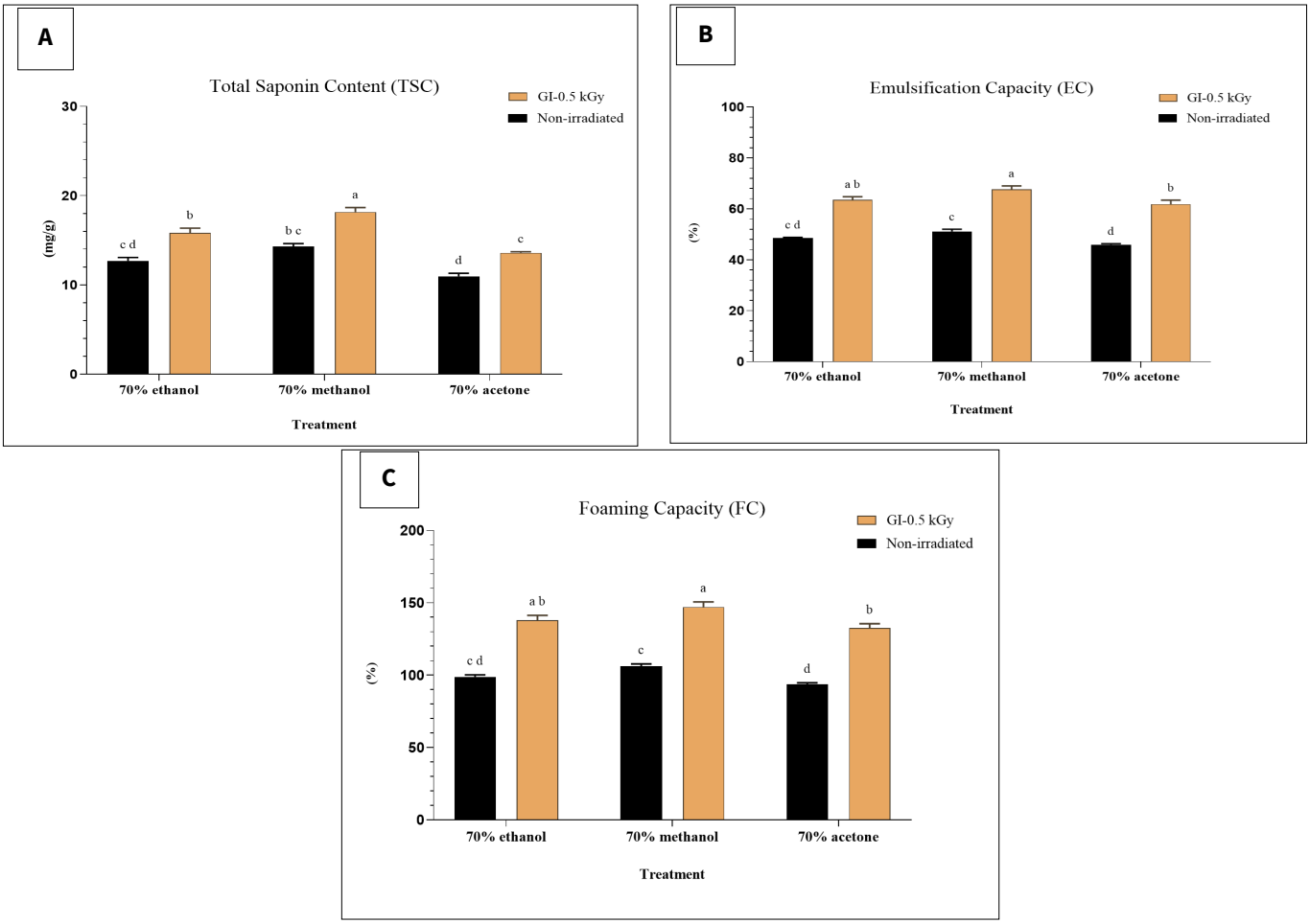

Fig. 2. Total saponin content and functional properties of okara extracts obtained from non-irradiated and gamma irradiated (GI-0.5 kGy) soybean seed: (A) total saponin content (TSC); (B) emulsification capacity (EC); and (C) foaming capacity (FC) of okara extracts prepared using different solvents. Values represent mean ± SE ($n = 3$). Different letters above bars indicate significant differences among treatments (two-way ANOVA followed by Tukey's HSD test, $p < 0.05$).

purity and that amplify interfacial adsorption (23). These studies consistent with mass transfer theory; the rate-limiting step in solid-liquid extraction is often diffusion through the solid matrix rather than solubility in the bulk solvent. Irradiation accelerates extraction kinetics uniformly across solvents, provided the saponins have sufficient solubility in each solvent system tested by increasing matrix porosity and reducing diffusion path length.

Effect of GI and solvent type on functional properties

Emulsification capacity (EC) differed significantly between irradiated and non-irradiated samples ($p < 0.05$). Irradiated samples exhibited consistently higher EC values across all solvent systems, accounts for 89.18 % of the total variation. The highest EC was observed in the irradiated samples extracted with methanol, followed by ethanol, while least EC was recorded in acetone extracts. Overall, GI resulted 31–35 % increase in the EC compared with non-irradiated controls (Fig. 2 B).

A comparable trend was observed for foaming capacity (FC), which increased by 1.39–1.42-fold across solvents in irradiated samples (Fig. 2 C). Gamma irradiation emerged as the dominant determinant, contributing 90.03 % of total variation, whereas solvent effects were secondary and consistent across treatments ($p = 0.909$).

Collectively, these results indicate that GI uniformly enhances saponin recovery and associated functional properties without altering solvent-specific trends. The observed improvements in EC and FC, particularly in 70 % methanol, can be attributed to irradiation-induced structural modifications of the okara matrix. In addition, the DS9422 soybean genotype contains a low level of KTI, potentially reducing structural stability and making proteins more susceptible to irradiation-induced cleavage of disulfide bonds and exposure of hydrophobic regions. Such modification promotes stronger interactions between protein and saponin, leading to the formation of saponin-dominant amphiphilic complex enhanced interfacial activity. These complexes stabilize oil-water and air-water interfaces via the Gibbs-Marangoni effect, forming viscoelastic films that resist droplet coalescence and foam collapse (24–26). The higher EC and FC observed for methanolic extracts are consistent with reports that high-polarity solvents improve the solvation of glycosylated saponins, thereby enhancing their surface activity (27).

Comparative studies functional properties have been reported for saponin derived from diverse botanical sources. For instance, quillaja saponin and protein–saponin complexes produce small droplet sizes, low critical micelle concentrations and strong interfacial stabilisation comparable to synthetic surfactants such as Tween® 20 and Tween® 80 (28). Triterpenoid saponins, including soyasaponins, exhibit strong foaming performance due to rapid interfacial diffusion and high surface elasticity (12, 29). The enhanced FC observed in the present study reflects increased availability of surface-active saponins and reduced surface tension facilitating greater gas integration during foaming process, thereby increasing foam volume and stability following irradiation.

The improved emulsifying and foaming properties have important technological implications. Such surface-active extracts could be applied as natural emulsifiers and foaming agents in oil-in-water dispersions such as beverages, salad dressings, sauces, gravies and plant-based mayonnaise and aerated foods including whipped toppings, confectionery foams, bakery items, dairy-free desserts and foam-stabilized beverages (12). Numerous studies have reported that saponin-stabilised emulsions achieve high physical stability and small droplet size comparable to synthetic surfactants, while also providing resistance to aggregation and creaming during storage. In case of protein-rich matrices, saponins interact with proteins to form mixed colloidal complexes that enhance film strength, a phenomenon reported for several plant-based systems (27). Furthermore, saponins enables simultaneous stabilisation of dispersions and encapsulation of lipophilic bioactive compounds, supporting applications in fortified beverages and nutraceutical foods. Additionally, saponins possess recognised bioactive properties, including cholesterol-lowering and antioxidant effects, suggesting their suitability for phytonutrients functional ingredients (29). Therefore, irradiated okara saponin methanolic extracts could serve as clean-label multifunctional ingredients in functional food system.

Explorative data analysis (EDA)

Exploratory data analysis was employed as a complementary analytical tier to move beyond the binary conclusions of hypothesis testing and to interrogate the underlying structure of the dataset. While sections 4.1 and 4.2 established statistical significance for the main effects of GI and solvent type on TSC, EC and FC, EDA was undertaken to reveal the degree of latent co-variation among these traits, to assess whether treatment separation is geometrically robust in multivariate space and to identify which treatment combination delivers the most comprehensive functional advantage. This distinction is critical: inferential tests confirm whether differences exist, whereas multivariate visualisation discloses how those differences are structured, scaled and interconnected dimensions that are invisible in univariate ANOVA outputs alone (30, 31).

Data quality and distributional characteristics

Replicate plots (Fig. 3 (A–C)) showed tight within-group clustering across all six solvent-irradiation combinations for TSC, EC and FC, with no evidence of outlier observations that could distort subsequent multivariate analyses. Coefficient of variation (CV) values were low across replicates, particularly for TSC, which is characteristic of vanillin–sulphuric acid methods applied under isothermal, controlled conditions. Notably, EC and FC exhibited slightly wider within-group dispersion than TSC, consistent with the intrinsic sensitivity of interfacial measurements to minor fluctuations in emulsion droplet history and air incorporation rate during vortex-induced foaming (12).

This differential replication precision across traits has a practical implication for interpreting the EC and FC results: the wider interquartile ranges observed in functional properties should not be interpreted as methodological artefacts but rather as reflective of the genuine physicochemical complexity of saponin -stabilised interfaces, where nanoscale structural heterogeneity in the adsorbed film propagates into macroscopic measurement variability (29). The high reproducibility of TSC, by contrast, indicates that the vanillin-sulphuric acid assay provides a stable compositional anchor from which EC and FC variations can be mechanistically interpreted. This replication quality assessment is particularly valuable in a dataset of limited dimensionality (three variables, six treatment groups, triplicate design), where a single outlier can disproportionately influence both correlation coefficients and PCA loadings (32).

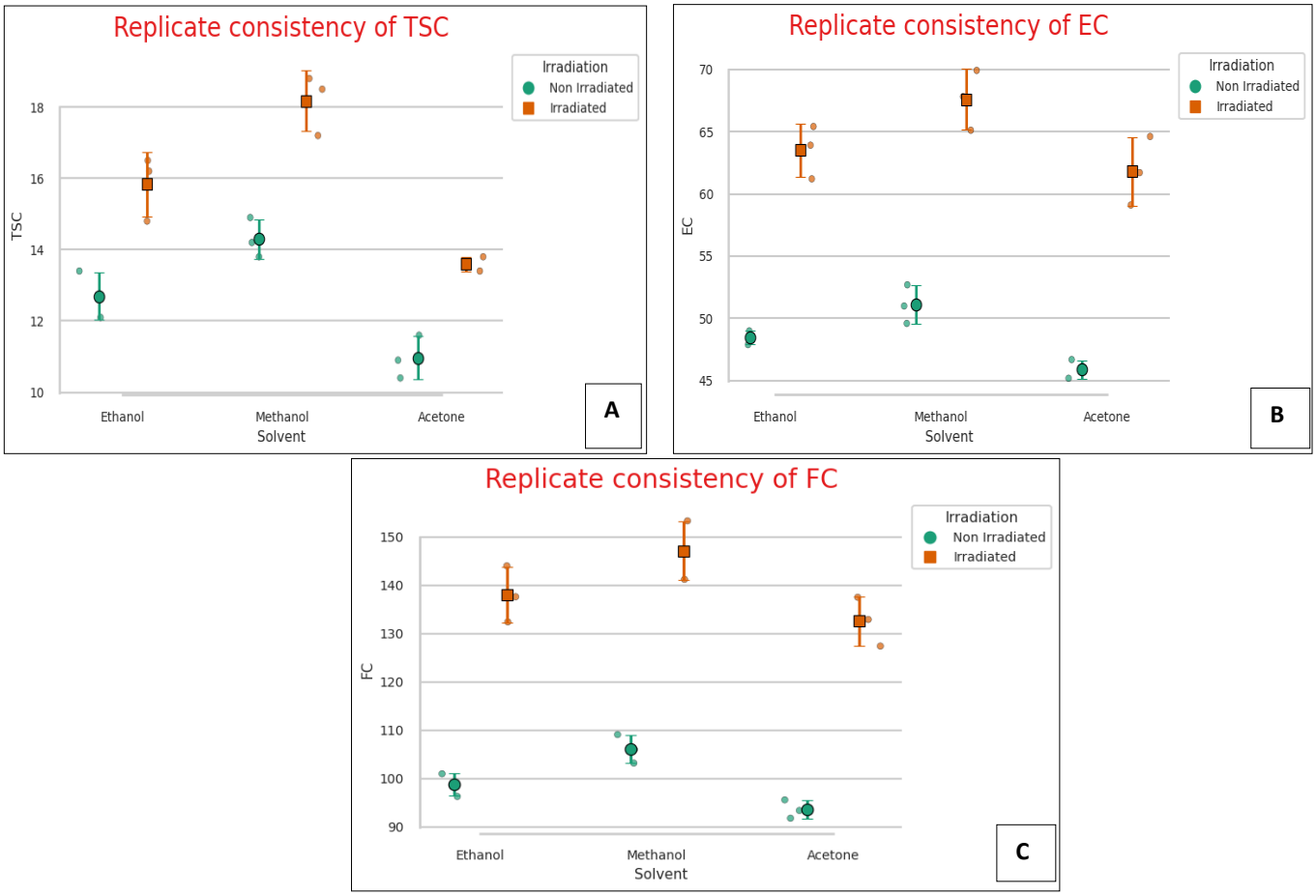

Fig. 3. Scatter plots showing replicate measurements of (A) total saponin content (TSC); (B) emulsification capacity (EC); and (C) foaming capacity (FC) for okara saponin samples obtained using different solvents from non-irradiated and gamma-irradiated (GI-0.5 kGy) soybean seeds.

Distributional symmetry and variance heterogeneity across treatment groups

Boxplot distributions (Fig. 4 (A–C)) revealed a consistent and diagnostically informative pattern: the irradiation-induced shift in median values was accompanied by qualitatively different variance profiles across TSC, EC and FC. For TSC, interquartile ranges were comparably narrow in both irradiated and non-irradiated groups, indicating that irradiation homogenised saponin recovery rather than introducing new sources of compositional variability. This variance-preserving enhancement is consistent with a mechanism operating at the matrix level irradiation uniformly restructures the okara cell wall polysaccharide network, making the saponin-binding sites equally accessible to the extracting solvent across all replicates (21).

By contrast, EC and FC showed noticeably broader interquartile spreads in irradiated groups relative to non-irradiated controls, particularly for methanol and ethanol extracts. This variance expansion in surface-active properties upon irradiation suggests that GI not only releases saponins from the matrix but also co-liberates structurally heterogeneous molecular populations including partially denatured proteins, modified polysaccharide fragments and saponin congeners with differing glycosylation patterns that interact differently with oil-water and air-water interfaces (32). The co-extraction of such molecularly diverse surface-active species would naturally introduce greater variability in measured functional outcomes while still producing a net improvement in median performance. This mechanistic interpretation is consistent with observations in irradiated legume protein systems, where low-dose gamma treatment produced compositionally richer, functionally superior but more heterogeneous protein extracts compared with untreated counterparts (27).

From a statistical governance perspective, the variance heterogeneity between groups for EC and FC is a meaningful observation that warrants attention beyond the Tukey-adjusted ANOVA framework applied in Section 4.2. Levene's test or Brown-Forsythe procedures, recommended for datasets with non-constant variance across groups, may offer additional sensitivity in detecting treatment-specific dispersion effects when functional interfacial properties are the primary response variables (24). Future investigations employing a broader dose range of GI treatments would benefit from explicit variance modelling to capture this heteroscedastic dimension of the response.

Interaction patterns (Irradiation × Solvent)

Interaction plots (Fig. 5 (A–C)) demonstrated near-parallel response trajectories across the three solvent systems for both irradiated and non-irradiated samples, visually confirming the non-significant treatment × solvent interaction reported in section 4.1 ($p = 0.311$). While the parallel patterns might initially suggest a trivially null interaction, it carries a substantive biological interpretation that extends well beyond statistical formality. True additivity between an GI and a solvent polarity implies that the two factors operate through mechanistically independent pathways that do not converge or interfere with one another a condition that is experimentally rare and practically valuable in process design.

In the context of the present study, additivity means that the irradiation-induced increase in saponin extractability and interfacial functionality is fully preserved regardless of which solvent is used and conversely, that the solvent-dependent performance hierarchy (methanol > ethanol > acetone) is maintained with identical rank ordering under both irradiated and

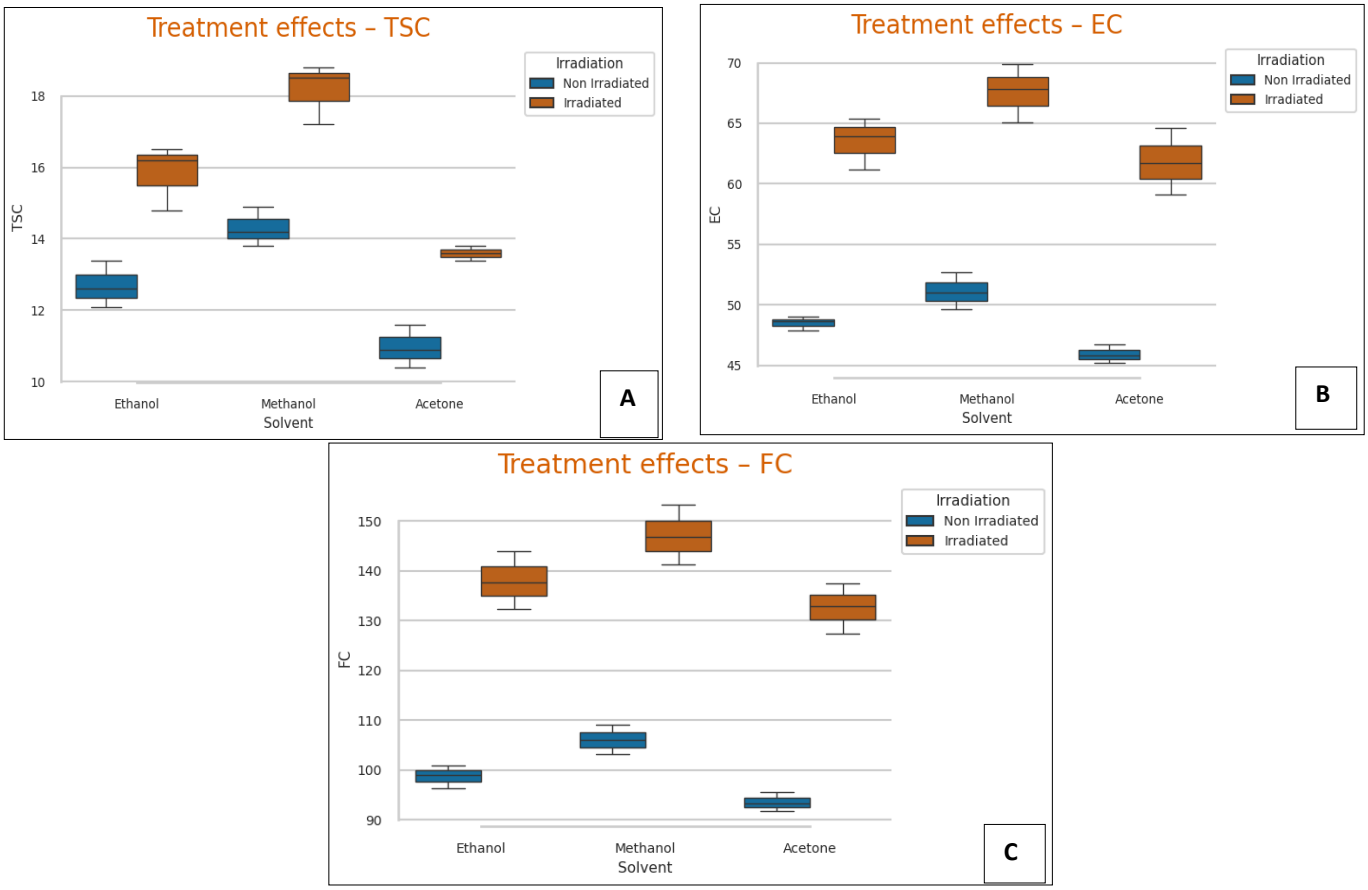

Fig. 4. Box plots illustrate the effects of irradiation and solvent type on (A) total saponin content (TSC); (B) emulsification capacity (EC); and (C) foaming capacity (FC). Non-irradiated and gamma irradiated samples were extracted using 70 % ethanol, methanol and acetone. Boxes represent the interquartile range with median values indicated by horizontal lines; whiskers show data dispersion ($n = 3$).

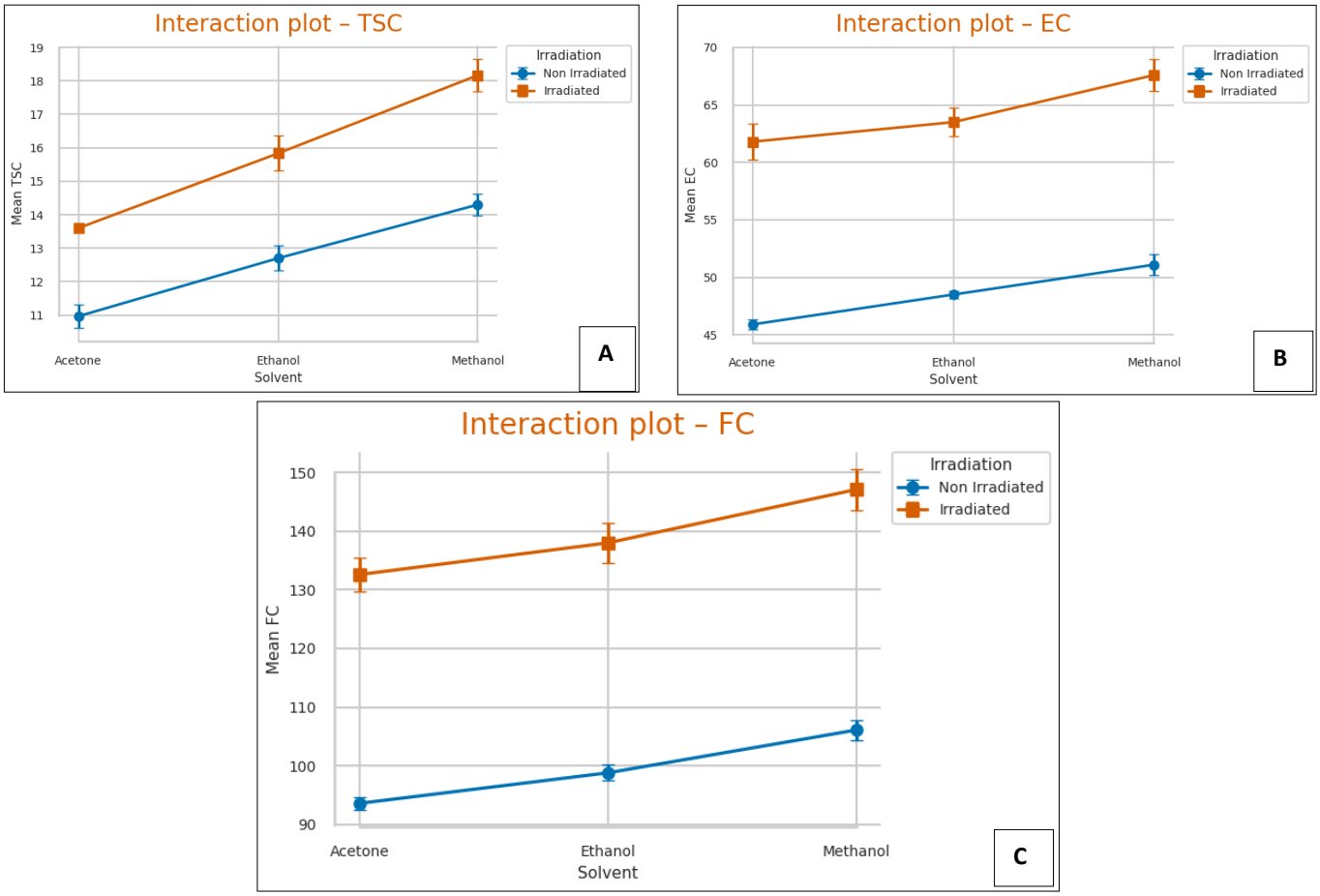

Fig. 5. Interaction plots depicting treatment-dependent changes in okara saponin content and functional properties. Plots illustrate mean ± SE, (n = 3) (A) total saponin content (TSC); (B) emulsification capacity (EC); and (C) foaming capacity (FC) in response to solvent type and gamma irradiation (GI-0.5 kGy).

non-irradiated conditions. This decoupling of effects has direct implications for extraction process scalability: an operator can independently optimise irradiation dose and solvent selection without antagonistic cross-interactions, a property that simplifies factorial process optimisation and supports implementation in continuous or semi-continuous extraction lines (33).

A further dimension of the interaction geometry is the consistent solvent rank ordering across irradiation levels, with methanol-based extracts yielding superior TSC, EC and FC relative to ethanol and acetone. This rank preservation indicates that solvent polarity acts as a fixed structural modifier of the extraction equilibrium, whilst irradiation acts as a kinetic accelerant of the approach to that equilibrium. The distinction between thermodynamic (polarity-governed) and kinetic (irradiation-governed) contributions to extraction performance is a conceptual framework supported by mass transfer modelling in solid-liquid extraction systems, where equilibrium solubility and effective diffusivity are influenced by distinct material properties of both the solvent and the matrix, respectively (34).

Structural co-variation among traits: correlation topology and network analysis

Pearson correlation analysis (Fig. 6 A) revealed strong positive pairwise associations among TSC, EC and FC, with correlation coefficients of r = 0.86 for both TSC-EC and TSC-FC pairs. The correlation network (Fig. 6 B) translated these coefficients into an edge-weighted graph in which EC occupied the central node with equidistant connections to both TSC and FC, reflecting its role as the functional bridge between compositional and surface-active parameters. This topological arrangement has a mechanistic rationale: EC is simultaneously governed by saponin surface coverage (linked to TSC) and by the viscoelastic stability of the interfacial film (linked to FC through shared dependence on saponin molecular architecture and surface diffusion kinetics).

The magnitude of the TSC-EC and TSC-FC correlations (r = 0.86) is significant in the context of saponin, where these relationships are frequently reported but rarely estimated across multiple extraction conditions simultaneously. Previous study noted that the functional properties of saponin scales non-linearly with concentration at low TSC ranges but approaches a linear plateau at intermediate concentrations typical of *n*-butanol-partitioned fractions, which is the range occupied by the extracts in the present study. The high linearity of the TSC-functional properties relationships observed here, therefore, suggests that the extracts operate within a concentration window that is kinetically favourable for interfacial adsorption neither too dilute for effective film formation nor too concentrated to cause steric crowding and interfacial desorption events that would diminish apparent EC and FC (27).

In the correlation analysis, no dominant difference was observed between TSC-EC and TSC-FC relationships, both functional properties correlated equally with TSC (r = 0.86), although EC and FC represent distinct interfacial phenomena. Emulsification requires lateral mobility of saponins at an oil-water interface, whereas foaming requires rapid adsorption at a rapidly

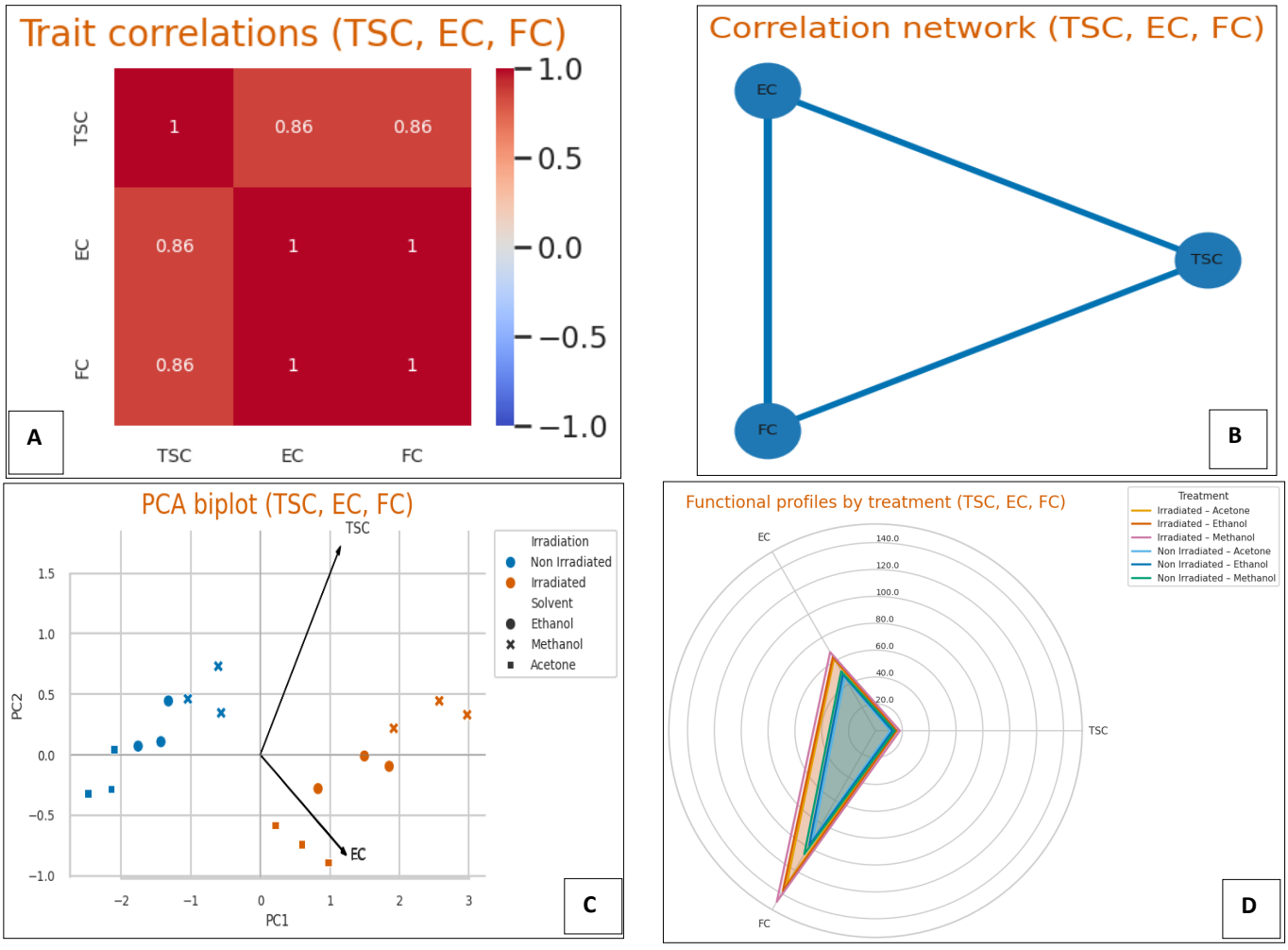

Fig. 6. Multivariate analysis of okara saponin extracts. (A) Pearson's correlation analysis; (B) correlation network; (C) principal component analysis (PCA) biplot; and (D) radar plots depicting relationships, sample clustering and treatment-specific functional profiles.

expanding air-water interface under dynamic shear conditions. The similar scaling of both processes with TSC suggests that the rate-limiting step for these interfacial parameters in this system is saponin availability, governed by extraction yield rather than molecular diffusion kinetics or conformational rearrangement at the interface (27). This finding supports the use of TSC as a reliable single variable for predicting interfacial functionality in irradiated okara saponin extracts.

Principal component analysis (PCA)

Principal component analysis biplot (Fig. 6 C) revealed that PC1 accounted for the dominant proportion of total variance, with all three variable loadings (TSC, EC, FC) projecting in the same direction and with similar magnitudes along this axis. The unidirectional loading pattern is the multivariate signature of a treatment factor that enhances all measured traits simultaneously and proportionally, precisely the behaviour characteristic of a matrix-level modification that releases the entire saponin functional package rather than selectively enriching a single functional attribute. This co-loading structure would not be expected if GI differentially affected saponin extraction without concomitant functional improvements, or if solvent type governed EC and FC through pathways independent of TSC accumulation. The geometric separation of irradiated samples at positive PC1 scores from non-irradiated controls at negative scores was complete across all solvent groups, with no overlap between the two irradiation clusters. This clean discrimination in multivariate space is stronger than what might be inferred from individual p-values alone, as PCA integrates the correlated variation across all three traits simultaneously to amplify between-group differences (35). The absence of inter-cluster overlap confirms that GI produced a consistent, multi-trait enhancement of a magnitude sufficient to be unambiguously resolved even in a low-dimensional PCA space, without requiring dimensionality reduction of a larger variable set.

Within the irradiated cluster, methanol-extracted samples consistently occupied the highest PC1 scores, followed by ethanol and acetone. This within-cluster stratification along PC1 indicates that the solvent polarity gradient modulates the magnitude of the overall functional package but not its compositional direction all irradiated extracts are functionally superior to all non-irradiated ones, but irradiated methanol extracts are the most superior within that group. The absence of meaningful PC2 separation by either irradiation or solvent type indicates that no second independent axis of functional differentiation exists within this experimental design, confirming that the two-dimensional treatment structure (irradiation × solvent polarity) is comprehensively captured by a single multivariate dimension. This finding has practical value: it implies that any future screening study evaluating GI-assisted saponin extraction from related soy by-products could reliably use a single composite functional index such as PC1 score as a dimensionality-reduced optimisation target, substantially reducing analytical burden (31).

The PCA results are also informative from a chemometric perspective. Hierarchical clustering applied to the PC scores would be expected to produce two primary clusters corresponding to irradiation status, with secondary sub-clustering by solvent type, reflecting the dominant and subordinate contributions of irradiation and solvent polarity to overall functional performance, respectively. This predicted clustering hierarchy is consistent with the variance partitioning reported in Section 4.2, where GI accounted for 90.03 % of the total variation in FC, shadowing the solvent contribution. PCA thus provides a geometric translation of the ANOVA variance decomposition into a spatial representation that reveals not only how much each factor contributes but how the factor contributions are distributed across the multi-trait functional space.

Integrated functional profiling

The radar chart (Fig. 6 D) provided a simultaneous, treatment-level comparison of all three parameters in a single polygon representation, enabling an assessment that is not accessible through individual bar plots or pairwise statistical comparisons. The polygon areas spanned a monotonic gradient from the smallest area (non-irradiated acetone) to the largest (irradiated methanol), with the four remaining treatment combinations occupying intermediate positions that tracked both the irradiation and the solvent polarity rank. This monotonic area gradient is a visually compelling representation of the joint operation of the additive factors identified in Section 4.3.3, irradiation and solvent polarity each independently expand the functional polygon and their co-application produces the maximum achievable area within this experimental design.

A particularly revealing feature of the radar geometry is the asymmetric polygon expansion between the EC and FC axes relative to the TSC axis as treatment conditions improve. Irradiated methanol extracts showed disproportionate extension along the EC and FC vectors relative to the TSC vector, indicating that the functional properties amplify to a greater relative degree than the compositional increase in saponin content alone would predict from a purely proportional relationship. This proportional functional gain is consistent with the concept of a critical interfacial concentration threshold, below which saponin molecules are too dispersed to form coherent, stable interfacial films and above which cooperative adsorption and film consolidation produce non-linear improvements in EC and FC (12, 25). The irradiation substantially increased TSC beyond this threshold revealing a qualitatively different interfacial properties regime in which saponin-protein complexes contribute synergistically to interfacial stability an effect that cannot be predicted from the linear correlation coefficients alone and that the radar geometry uniquely captures.

The radar outline introduced a transparent, multi-criteria decision tool for selecting optimal extraction conditions when multiple functional parameters must be balanced simultaneously. Unlike single-objective optimisation based on TSC or EC alone, the polygon area metric integrates all three parameters' dimensions and can be extended to incorporate additional quality parameters such as saponin purity, quantities, colour and bitterness threshold in future product formulation studies targeting food-grade emulsifier applications. These findings collectively position GI treated, polarity-optimised extraction as a mechanistically coherent and practically scalable strategy for the valorisation of okara as a source of natural surfactants and phytonutrients. Overall, this EDA pipeline situates the results within a contemporary data-driven and ML-oriented framework for food systems, where unsupervised multivariate tools are repeatedly used to reveal the structure-function relationships prior to predictive modelling (36, 37).

Conclusion

This study shows that GI at a dose of 0.5 kGy significantly enhances total saponin recovery from okara across solvent systems, with 70 % methanolic extraction yielding the highest content, approximately 27 % higher than that of non-irradiated samples. The resulting fractions exhibited substantial improvements in functional performance, with emulsifying capacity increasing by 31–35 % and foaming capacity by 1.39–1.42-fold, attributable to irradiation-induced structural modification of the okara matrix that increases the availability of surface-active saponins. Chemometric mapping developed in this study confirmed strong co-variation among total saponin content and functional properties and clearly distinguished irradiated from non-irradiated samples. Together, these findings indicate that combined use of GI and polarity-optimized methanolic extraction, supported by chemometric analysis, is an effective approach for recovering saponins from soy-processing by-products and for utilising okara as a source of natural surface-active phytonutrient ingredients for food, pharmaceutical and related applications within a circular bioeconomy framework.

Future research should focus on process scale-up, validation in real food matrices, characterisation and quantification of individual saponin fractions and safety evaluation to support industrial application and regulatory acceptance. Additionally, the chemometric mapping developed in this study provides a basis for future optimisation of colloidal stability and functionality using advanced data driven and machine-learning approaches.

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Authors' contributions

RL was involved in investigation, data curation, formal analysis, validation, visualization, cover image and writing of the original draft. DM contributed to investigation, data curation, formal analysis and manuscript review. KPK was responsible for statistical analysis, EDA analysis, data curation, reviewing and editing. ASR, MD, AR participated in investigation and data curation. MR provided essential resources for the study and SK contributed to investigation. AD was responsible for conceptualisation, methodology, supervision, review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the the author(s) used generative AI tools (ChatGPT) in order to assist with language editing, improving clarity and readability, restructuring sentences for academic style. All scientific content, data interpretation and conclusions were developed by the authors. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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