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Research Article

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Posted Date: August 19th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7118366/v1>

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Additional Declarations: No competing interests reported.

**Synergistic Ultrasonication-Enzymatic Strategy for Bioactives Extraction from Jamun
(*Syzygium cumini*) Pulp for Functional Foods Application**

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Abstract

Conventional pasteurization used for the elimination of harmful microorganisms also leads to thermal degradation of heat-sensitive bioactive composition present in Jamun (*Syzygium cumini*) fruit, thereby reducing the antioxidant activity. To address this limitation, the research focuses on how advanced technology, Ultrasonication Assisted Enzymatic Treatment (USEZT) has enhanced bioactive and biomacromolecule contents of jamun pulp. The study also has the potential to industrialise most perishable and sensitive jamun fruit pulp. Application of Response surface methodology (RSM) helped to capture the treatment effects and further ANOVA validated the predictive model's robustness (R^2 value). The optimized condition: 25 min sonication time, 20% amplitude, 0.45% enzyme concentration, and 60 min incubation gave experimentally measured values very close to model predictions, confirming the approach.

Ultrasound-accelerated cell wall breakage and enzyme-catalysed hydrolysis promoted retention of phenols, Acn and enhanced antioxidant capacity, though with prolonged exposure, inducing oxidative degradation. Physicochemical analysis at optimized conditions increased: juice yield ($75.5 \pm 0.07\%$), ascorbic acid (24.91 ± 0.42 mg/100g), titratable acidity (1.38 ± 0.05 %), total phenolic content (TPC) (760 ± 0.03 mg/100g), ferulic acid (0.91 mg/100g), gallic acid (42.10 ± 0.05 mg/100g), Quercetin (0.80 ± 0.05 mg/100g), Acn (38.57 ± 0.10 mg/100g), increased redness (a^*) (31.29 ± 0.02) and colour density (2.30 ± 0.09). The low-cost, non-thermal ultrasonication-enzymatic method keeps heat-sensitive bioactives in Jamun pulp intact, improving its nutritional value. This eco-friendly method helps maintain nutrient balance and supports targeted delivery in functional food applications.

Keywords: Ultrasonication, Enzymatic treatment, Pectinase, Phenolic content and Acn

Abbreviations

AR: Analytical Reagents, °C: Degree Celsius, KHz: kilohertz, mL: Millilitre, W: Watt, ANOVA: Analysis of Variance, gm: Gram, NaOH: Sodium Hydroxide, mg: Milligram, H₂SO₄: Sulfuric acid, min: Minutes, DPPH: 2,2- diphenyl-1-picrylhydrazyl, mPa.S: Millipascal second, mg: Milligram, g: Gram, µg: microgram, HPLC: High Performance Liquid Chromatography, ABTS: 2,2' – Azino- bis (3- ethylbenzothiazoline- 6 – sulfonic acid, TSS : Total Soluble Solids, TS: Total Sugars, RS: Reducing Sugars, TRS: Total Reducing Sugars, TV: Titre Value, TPC: Total Phenolic Compounds, Pvt Ltd: Private Limited, µL: Microliter, nm: Nanometre, HCL: Hydrochloric acid, N: Normality, OD: Optical Density, Incb: Incubation, Enz Con: Enzyme concentration, Incb tm: Incubation Time, Mm: Millimolar, g: grams, pH: Potential hydrogen, Fig: Figure, USEZT: Ultrasonication Assisted Enzymatic Treatment, Enz con: Enzyme Concentration, Acn: Anthocyanin, S: seconds, Adq: Adequate, TSS: Total soluble solids, CD: Colour Density, SD: Standard Deviation, hrs: hours

1. Introduction

Jamun is a tropical underutilized fruit belonging to the Myrtaceae family. It is an abundant source of phytochemicals (polyphenols, ascorbic acid, Acn) compounds of pharmacological importance. Bioactive constituents present in jamun, like phenolic acids, anthocyanins (Acn), and terpenes, are reported to possess significant health effects, viz., anti-inflammatory, antioxidant, anticancer, antimicrobial and hypoglycemic activity. Phenolic acids prevent oxidative stress and improve sensory characteristics, Acn reinforce cellular defence, and terpenes prevent infections and manage blood glucose [1, 2, 3, 4, 5, 6]. The fruit has been traditionally consumed as a medicinal drink by mixing it with water and preservatives, such as sugar and citric acid, to extend its lifespan. Further to increase the palatability, the fruit has been soaked in brine solution to reduce its astringent flavour [7]. One major concern associated

with the jamun fruit is its shelf life. Properly matured jamun fruits are highly perishable, which leads to over ripening, excessive tissue softening and becomes prone to pathogenic attacks (*necrotrophy Brytis cinerea*) leading to a significant postharvest loss. Whereas jamun can be stored at room temperature (35 to 45°C) for 2 days and at low temperature the shelf life increases up to 20 days [8]. Traditionally fruit pulp has been extracted using mechanical press (Cloth, screw and belt press), decanter type centrifugation, thermomaceration and diffusion extraction. Later on, the need for microbially stable jamun based products lead to the development of conventional processing methods like sterilization and pasteurization where microbial stability was achieved at the loss of bioactive compounds like polyphenols, Acn and ascorbic acid which leads to structural changes in aromatic compounds, unpleasant smell and discoloration in fruit pulp [7, 9, 10, 11]. The unorganized cultivation and harvesting methods also lead to a substantial annual loss of jamun fruit. Innovative new processing technique is frequently undertaken to improve the value of fruit pulp by addition of preservatives, development of new processed product and higher consumer acceptability. To overcome the problem techniques such as ultrasonication and enzymatic treatments has been used in the study.

Enzymatic treatment was found to be a better option to conventional methods in comes to pulp retrieval. Liquefying enzyme pectinase is a mixture of polygalacturonase-heterogeneous, pectin lyase-heterogeneous, and pectin methyl esterase-heterogeneous. Pectinase hydrolyse cell wall polysaccharides in the fruit through disruption of glycosidic bonds, cell softening, and facilitation of juice release [12,13, 14, 15, 16]. Additionally enzymatic treatment of pulp prevents the destruction of valuable fruit constituents and promotes hydrolysis of pecto-cellulosic cell wall structural components efficiently to facilitate easier release of valuable compounds [17, 14, 18]. But the process of extraction was labour intensive and time taking. Whereas Ultrasonication (US), helps in better cell disintegration and is considered better

replacement to traditional thermal techniques due to the ability to extract target compound at a lower processing time and temperature with minimal damage to the nutritional profile of food product [19]. It was reported that the energy applied during sonication results in the release of charged molecules from the complex mixture and exhibits substantially higher electrical conductivity compared to the samples subjected pasteurization. The conductivity difference implies that pasteurization accounted for the degradation of some compounds whereas sonication, on the other hand, primarily enabled the release of molecules without any degradation [20, 21].

The originality of the study is based on water based green technology where no harmful solvents were used. The method is energy-sufficient, and environmentally friendly, minimizing the use of chemical additives or thermal processing. The research uncovers key points of degradation, where extended sonication adversely impacts TPC, Acn, and antioxidant activity. Lower polydispersity index and enhanced zeta potential show that the improved colloidal and pulp stability, preventing sedimentation and supporting suitability of jamun pulp in nutraceutical and pharmaceutical uses in future. Using response surface methodology (RSM), the research maximizes the water-based extraction process to recover bioactive compounds and reduce wastage. Unlike previous research where only enhanced extraction is focused for processed fruit juices/pulp, the present study gives a precise equilibrium between enhancement and degradation, which is very much applicable to industries [22, 14]. The balance between sonication time and amplitude, demonstrating that excessive sonication leads to the degradation of phenolic compounds through excessive reactive oxygen species generation. This combined system exploits the mechanical properties of ultrasonication in the disruption of cell walls for the improvement of enzyme accessibility and activity, generating improved yields of the target compound. The study determines the equilibrium of maximum extraction and degradation, which is one that has been left out in previous studies. This makes it suitable for clean-label,

natural, and functional drinks, fitting the current industry trends in food technology. The bioactive obtained through optimized treatment can find applications such as healthy food ingredients, functional foodstuffs, plant based supplements and herbal medicines which can be added to serums, and products to promote skin health and retard premature aging. The Acn, antioxidant dense preservatives, present in jamun also act as natural colorants and could be used as a cleaner-label option over synthetic additives.

2. Materials and Methodology

2.1 Chemicals and Reagents

Chemicals used in the study include Sodium hydroxide, ascorbic acid, oxalic acid, phenolphthalein, 2,6-dichlorophenol indophenol, Fehling A, Fehling B, methylene blue, copper sulphate, sodium sulphate, hydrochloric acid, sulfuric acid, Folin Ciocalteu, sodium carbonate, gallic acid, α galactosidase, pectinase, ethanol, methanol, charcoal, DPPH, 2,2'-ABTS, pectinase (Pectinex Ultra SPL), potassium persulfate, trolox, potassium ferrocyanide, quercetin, trichloroacetic acid and ferric chloride. The chemicals were analytical grade and had been procured from the Sigma and Aldrich (Mumbai, India) company.

2.2 Jamun pulp preparation

Jamun fruits of 'Ra' variety, fully matured, free from any visible defects, were collected from local farmers of village Hardaspur, Phagwara, Punjab during the harvest period May to July (2024). The selected fruits were washed three times in tap water and then a single wash was done using distilled water. Further, the pulp (1000 mL) was manually separated from the seeds to avoid any bitterness and homogenized using a mixer (Longway Super Dlx 750W) for 5 min to achieve a uniform consistency. The blended mixture (Improvhome stainless steel filter) was strained to remove bulk suspended particles and to achieve an even consistency. Filtrate was subsequently pasteurized (60°C for 2 min) in water bath followed by instant

cooling in cold water bath and the mixture was stored at -16°C in a 300 mL capacity glass bottles until required. The pasteurized pulp sample (60 mL pulp + 20 mL distilled water) was taken as control. For extraction process a 20 KHz prob sonicator (Touch Screen Probe Sonicator With Sound Proof Enclosure - PRO1200, LABMAN with standard prob of 6 mm, 12 to 1200W) with 100 mL capacity was used. The pulp samples (60 mL pulp + 20 mL distilled water) were sonicated under the amplitude setting of 70%, with power of 400 W and pulse duration for 5 seconds on and off at 20°C temperature. Keeping the temperature at 20°C is crucial to avoid overheating because ultrasonication produce large amounts of heat through cavitation and to avoid this the sample beaker was submerged in a vessel of ice-water for 10 to 15 min for active cooling. Another way was rather than repeating sonication, the probe was pulsed (e.g., 10 s on, 10 s off). This allows the sample time to recover between bursts. In the second phase of extraction sonicated samples were subjected to enzymatic treatment with pectinase (3.5–7 units/mg activity) at an incubation temperature of 45°C in a hot water bath.

2.3 Experimental design

Box Behnken design, a type of RSM (Design-Expert 8.0) utilized in the study is a three-level factorial design similar to central composite designs, but without extreme treatment combinations [23]. The independent variables in BBD were assigned three levels (-1, 0, 1), and the entire experimental setup included 29 trials, with five central points at the centre points [16]. The four independent variables used in the experimental design include X_1 - ultrasound time: 10 to 30 minutes, X_2 - ultrasound amplitude: 20 to 40%, X_3 - enzyme concentration: 0.03 to 2% w/v and X_4 - incubation time: 30 to 60 minutes. For evaluation of the effect of the linear, quadratic, and interaction effects between the process variables, a second-order model formulated based on Response Surface Methodology (RSM) was used [21]. The statistical significance of every response was identified using Analysis of Variance (ANOVA)

[24]. The accuracy, adequacy and goodness of fit of the model was tested using multiple statistical parameters, such as the coefficient of variation (CV), coefficient of determination (R^2), adequate precision and adjusted R^2 .

2.4 Physicochemical analysis of jamun pulp

2.4.1 pH

pH values of the pulp samples were analyzed by a pH meter (ETS–D6A), which was calibrated with pH 4.0 and 7.0 phosphate buffer solutions to obtain precise values [25].

2.4.2 TSS

TSS of the samples was tested with a refractometer (ERMA INC, Tokyo Japan). The measurement range of the refractometer was 0 to 90 °Brix. Prior to each measurement, the refractometer was calibrated using distilled water to ensure accuracy [23].

2.4.3 Viscosity

Viscosity of the pulp samples were estimated at room temperature (around 28 ± 1 °C) in an Ostwald viscometer, $20 \times 10 \times 10$ cm (Borosilicate FZN Glassware, Mumbai, India). It was described as millipascal-seconds (mPa s) [26, 23].

2.4.4 Moisture content

Approximately 2 g of pulp sample was dried at 105°C for 2 hours [27] and the moisture content, was calculated using a standard formula based on the weight loss during the drying process.

$$\text{Moisture content} = \frac{Q_0 - Q_1}{Q_0} \times 100 \% \quad \text{Eq. 1}$$

Q_0 and Q_1 represents sample weight (g) before and after drying (g)

2.4.5 Ash content

Ash content was analysed by controlled incineration in a muffle furnace. Initially the crucibles were preconditioned by heating at 600°C for 30 min. Subsequently, 2 g of samples were weighed into the pre-tared crucibles and incinerated at 600°C until consistent weight or light grey ash was achieved, and the process required 6 hours [28]. After cooling, the final weights were recorded, and the ash content was calculated using a standard formula.

$$\text{Ash content} = \frac{Q_2 - Q_0}{Q_1 - Q_0} \times 100 \% \quad \text{Eq. 2}$$

$$Q_1 - Q_0$$

Q_0 , crucible weight (g)

Q_1 , Wg of sample in the crucible (g)

Q_2 , Wg of ash in crucible (g)

2.4.6 Juice yield

200 g of jamun pulp was filtered using a multi-layered muslin cloth [23]. The filtrate was transferred to a measuring cylinder to find the final volume. Yield was calculated as follows:

$$\text{Juice yield (\%)} = \frac{A_1}{A_2} \times 100 \quad \text{Eq. 3}$$

$$A_2$$

A_1 = Filtrate volume (mL)

A_2 = Weight of jamun pulp (g)

2.4.7 Total titratable acidity

For titration 5mL of pulp sample were diluted with 10 mL distilled water. Further to remove natural pink tinge of the jamun pulp and facilitate the titration procedure 1 gm of charcoal was used and charcoal remains from sample was separated through a Whatman No. 1 filter paper.

The mixture was titrated against 0.1N NaOH and 2 drops of phenolphthalein indicator was added to the mixture to mark the end point of titration [29, 30]. Titratable acidity formula:

$$\text{Titratable acidity} = \frac{(0.1\text{N}) \text{ NaOH} \times \text{Volume of NaOH} \times \text{equivalent weight}}{\text{Volume of sample}} \quad \text{Eq. 4}$$

2.4.8 Ascorbic acid

For ascorbic acid determination the pulp sample was treated 0.4% oxalic acid and further analysed by titration with 0.04% solution of 2,6-dichlorophenol indophenol dye. A standardized dye solution was prepared from L-ascorbic acid (0.1 mg/mL). For sample extract, 5 mL of the jamun pulp was homogenized with 0.4% oxalic acid, and diluted of final volume of 100 mL. From the extract, 10 mL of the filtered was titrated against the standardized dye until a persistent pink colour lasted for at least 20 seconds, showing the endpoint of the titration [31]. The amount of ascorbic acid was found by applying the following:

$$\text{Ascorbic acid content (mg/100g)} = \frac{\text{TV} \times \text{dye factor} \times \text{volume make up} \times 100}{\text{aliquot volume} \times \text{sample weight}} \quad \text{Eq. 5}$$

2.4.9 Total protein

The content of protein was analysed as per the Kjeldahl technique outlined by [32], Jamun pulp was transferred to digestion flask to which copper sulphate (0.2g), sodium sulphate (10 g) and H₂SO₄ (2.5mL) were added. The mixture was heated for 1 hour until sea green appeared, indicating the completion of digestion process. After digestion 200ml of distilled water and 2 drops of phenolphthalein indicator was added to the sample. Further 50% NaOH solution was added slowly along the flask's inner wall until the colour change from green to brown. In a separate beaker 50 mL of 0.1 H₂SO₄ solution followed by few drops of methyl red indicator was mixed and the distillation process was then carried out. The distillate was titrated with 0.1 N NaOH. The formula for total protein content:

$$\text{Protein content} = \frac{\text{Blank TV} - \text{sample TV} \times 1.4 \times \text{normality of NaOH} \times 6.25}{\text{Eq. 6}}$$

233 Sample weight

234 TV = Titre value

235 **2.4.10 Total dietary fibre**

236 Fibre content was estimated by enzymatic gravitation procedure. 0.5 mL of sample dried at 60
237 °C for 15 hours was washed with 80% ethanol. Further enzymatic treatment was performed: α
238 amylase (incubated at 90 to 100°C, 30min) for gelatinization and hydrolysis of starch into
239 dextrin's. This was followed by protease enzyme (incubation at 60°C, 30 min) to break down
240 protein and in the last stage of enzymatic digestion where amyl glucosidase enzyme broke
241 down dextrin to glucose and the indigestible part was precipitated by addition of 95% ethanol.
242 The precipitate fibre was collected via filtration and the remaining residues was dried and
243 weighed. Non fibrous compounds, protein and ash was quantified by Kjeldahl method and
244 through incineration by muffle furnace [33]. Formula for dietary fibre content:

$$245 \quad \text{Total dietary fibre} = \frac{\text{Residual Weight} - \text{protein} - \text{ash} - \text{blank}}{\text{Sample Weight}} \quad \text{Eq. 7}$$

246 Sample Weight

247 **2.4.11 Total sugar and reducing sugar**

248 For analysis 2 mL jamun pulp was diluted with 250mL of distilled water. Additionally, 10 mL
249 aliquots of Fehling's solutions A and B were pipetted into a separate conical flask. The mixture
250 was slowly boiled over hot mental for 2 min. Then methylene blue indicator (1 mL) was added
251 to the solution without stopping the boiling process. While the mixture was again brought
252 to boiling point, the normal dextrose solution was added gradually until the blue colour of the
253 indicator had vanished. The titre value was also noted as the volume of dextrose
254 solution required to completely reduce the copper in 10 mL of Fehling's solution [34,32].

255 For inversion of sugar solutions 50 mL aliquot was mixed with 10 mL of hydrochloric acid in
256 a volumetric flask and stored at room temperature for 24 hours. After the inversion process,

phenolphthalein indicator was employed to neutralize the solution with concentrated sodium hydroxide, and the resulting solution was diluted to a final volume of 100 mL. The inversion degree was quantified using a titration with 25 mL of Fehling's solution, mixed with Fehling's solutions A and B, to determine the presence of invert sugar as expressed by the total sugar content. RS and TRS can be calculated as:

$$RS (\%) = \frac{\text{invert sugar (mg)} \times \text{volume made up (mL)} \times 100}{TV \times \text{Sample volume} \times 1000} \quad \text{Eq. 8}$$

$$TV \times \text{Sample volume} \times 1000$$

$$TRS (\%) = \frac{\text{invert sugar (mg)} \times \text{final volume made up (mL)} \times \text{original volume (mL)} \times 100}{TV \times \text{sample volume} \times \text{sample aliquot for inversion} \times 1000} \quad \text{Eq. 9}$$

$$TV \times \text{sample volume} \times \text{sample aliquot for inversion} \times 1000 \quad \text{Eq. 9}$$

$$TS = (TRS - RS) \times 0.95 + RS$$

2.4.12 Assessment of bioactive compounds and antioxidant activity

2.4.12.1 Detection of TPC

For analysis 2 mL of pulp sample was mixed with 8 mL of 80% methanol and centrifuged (4000 × g) for 20 min. Then, 50 µL of the supernatant was added with Folin-Ciocalteu reagent (100 µL) and of deionized water (1500 µL). The solution was kept in room temperature for 10 min before being added with 50 µL of a 20% sodium carbonate solution. The reaction mixture was incubated in the dark for 2 hours and absorbance was measured at 765 nm [27].

2.4.12.2 Total Acn

Acn was determined using spectrophotometric method. Ethanolic HCl solution made by mixing 85 mL of 95% ethanol with 15 mL of 1.5N HCl was added to the fruit pulp for the sample preparation [31, 35]. After ensuring stability for 30 min in a dark atmosphere,

absorbance measurements were made at 530 nm, and the mathematical formula used to determine the total Acn content was:

$$\text{Total OD/100mL} = \frac{\text{Sample OD} \times \text{Volume made} \times 100}{\text{Sample volume}} \quad \text{Eq. 10}$$

$$\text{Total Acn} = \frac{\text{Total OD/100mL}}{98.20} (\text{mg/100mL}) \quad \text{Eq. 11}$$

98.20

2.4.12.3 DPPH scavenging activity

2 mL pulp sample mixed with 2mL DPPH solution was incubated in room temperature for 30 min and the absorbance was measured at 517nm. The same setup was addressed to blank using ethanol. The decrease in absorbance reading caused by the proton donating activity was monitored with spectrophotometer at 517nm [36]. Equation for determining DPPH scavenging activity:

$$\text{DPPH scavenging activity (\%)} = 100 \times \frac{Q_0 - Q_t}{Q_0} \quad \text{Eq. 12}$$

Q_t , absorbance of pulp

Q_0 , absorbance of control

2.4.12.4 ABTS Radical scavenging activity

A freshly prepared ABTS stock solution prepared by combining 7 mM ABTS solution and 2.45 mM potassium persulfate solution was used to assess ability of pulp samples to scavenge ABTS radicals. The stock solution was kept aside in dark for 16 hours before being used. Further 500 μ L of diluted ABTS solution (absorbance of 0.7 ± 0.02 at 734 nm) was added to the pulp

sample. The absorbance of test sample and control was measured at 734nm after 6min of incubation [37, 16]. Formula for ABTS radical scavenging activity:

$$\text{ABTS activity (\%)} = (1 - \frac{A_1 - A_2}{A_1}) \times 100 \quad \text{Eq. 13}$$

A_1

A_1 , Absorbance of the ABTS solution in control sample

A_2 , Absorbance of the ABTS solution in the sample

2.4.12.5 Reducing power

In this assay, 1 mL methanolic extract at four concentrations was added to 2.5 mL of 1% potassium ferrocyanide and 2.5 mL of 0.2 mM phosphate buffer (pH 7.4) and the solution was further mixed with 2.5 mL of 10% trichloroacetic acid and incubated for 25 min. After incubation the solution was centrifuged (1200 g for 10 min) and 2.5 mL of the supernatant from then added to 0.5 mL of a 0.1% ferric chloride solution and an equivalent volume of distilled water. After measuring the resultant absorbance at 700 nm, the reducing power was calculated using a standard curve [38].

2.4.13 Colour

The colour characteristics, of samples were examined using Hunter colorimeter. For analysis a 20 mm glass optical cuvette with a light passage was filled with 50 mL of the sample. In colorimeter lightness is indicated by L value, where a^* stands for the positive and negative colour components of red and green axis, respectively, and b for the positive and negative colour components of yellow and blue axis, respectively. L, a and b values between control and processed pulp samples were compared to assess colour modifications in the pulp samples [39] and to evaluate magnitude of colour changes formula this has been used.

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad \text{Eq. 14}$$

323 L, a, b corresponds to colour value of control sample

324 L_0 , a_0 and b_0 represents colour value of treated samples.

325 **2.4.14 Colour Density**

326 The colour density was quantified using a microplate reader. A 200 μ L aliquot of the sample
327 was loaded into the reader to measure the absorbance at 420, 510, and 700 nm [39]. Test
328 samples were taken in triplicate. Formula for colour density:

$$329 \quad \text{Colour Density} = [\text{OD (510)} - \text{OD (700)}] + [\text{OD (420)} - \text{OD (700)}] \quad \text{Eq.15}$$

330 **2.4.15 HPLC analysis of phenolic compounds**

331 HPLC determination of the phenolic compounds i.e. gallic acid, ferulic acid and quercetin
332 was performed using a reversed-phase column C_{18} of length 250 mm $\times$ 4.6 mm, 5 μ m (Agilent
333 1260 Infinity, Chennai) at temperature of 25°C. The HPLC system with UV/Vis- DAD diode
334 array was used in quantitation of different polyphenolic compounds at different wavelengths
335 depending on the maximum absorbance. To purge impurities, a guard column (4 $\times$ 4 mm; 5
336 μ m) was placed in front of the column used for the main analysis. The mobile
337 phase contained three solvents: Solvent I (0.1% formic acid water, v/v), Solvent II (0.1%
338 formic acid in acetonitrile), and Solvent III (0.1% formic acid water). The mobile
339 phase flow was at 1.0 mL/min, while that of injection of standard solutions and extraction
340 samples was to 2.0 μ L. The chromatographic analysis conditions were referred from the report
341 by [40] with slight modifications.

342 **2.4.16 Particle size characterization**

343 Pulp samples diluted with (pH 7.4) phosphate buffer in 1:10 ratio were further were
344 centrifugated at 6000 rpm (15 min), the soluble fraction was removed to analyse the particle
345 size. The samples were further sonicated with probe sonicator. For the final formulation, the

samples were sonicated at 5 °C, with 5-second pulses repeated over a period of 15 minutes. After preparation, the samples were placed in glass vials at 25 °C for further analysis by a computer-based particle size analyser (Zetasizer Nano ZS, Malvern Instruments Ltd., Malvern, UK) [41].

2.4.17 Sensory analysis

Ten novice judges performed the sensory analysis of chilled jamun pulp and the panel has been trained for how to use hedonic scale to assess sensory characteristics of the sample on a scale of 1 (strongly dislike) to 9 (strongly like), taking into account sensory parameters of pulp i.e. flavour, colour, odour density and overall impression prior to the evaluation. Five differed pulp samples have been served for analysis purpose. The samples taken for sensory analysis were C: Control sample (without treatment), USEZT treated JP1: Jamun Pulp 1 (sonication- time 25min, Amplitude - 20%, Enz con- 0.45%, Incb tm- 60min), JP2: Jamun Pulp 2 (sonication- time 20min, Amplitude - 30%, Enz con- 1.01%, Incb tm- 45min), JP3: Jamun Pulp 3 (sonication- time 20min, Amplitude - 30%, Enz con- 0.03%, Incb tm- 30min), JP4: Jamun Pulp 4 (sonication- time 20min, Amplitude - 30%, Enz con- 1.01%, Incb tm- 60min) (Table 1). The treated samples were selected on the basis of combination with better optimization results. The samples were served in 100 mL paper cups and the evaluation was conducted at room temperature. The findings for each attribute were averaged to establish the product's overall acceptability.

2.4.18 Statistical analysis

All the physicochemical and optimized treatment conditions were performed in triplicate and the results were noted as the mean $\pm$ SD with statistical significance $p < 0.01$. The results were analysed by ANOVA in SPSS 23.0 software (Chicago, IL, USA). Duncan's multiple range test

was utilized to separate means and the statistical variation were verified using a significance level of $p < 0.05$.

3. Results and Discussions

3.1 Regression analysis RSM model

Summary of ANOVA as presented in Table 2 shows the outcome of the responses according to the Box Behnken Design. The final regression models for the three responses were assessed by using the design software, as mentioned in equation 1, 2 and 3 in terms of significant coded factors for TPC, Acn and DPPH activity which has been used to predict the desired result. Validation of experiments were also carried out to confirm the predicted responses. Independent variables, and their corresponding coded levels, are mentioned in Table 1.

3.1.1 Impact of UAET on TPC of jamun Pulp

USEZT treatment resulted in substantial rise in TPC in comparison to control pulp and based on the regression model (Eq. 1) TPC of treated pulp sample range from 661 to 774mg/100g and the peak value for TPC was attained at treatment conditions: sonication for 20 min at 30% amplitude followed by enzyme concentration 1.01% at an incubation time of 45 min Table 1. The interaction terms exhibited a linear trend in relation to TPC, as illustrated in Fig. 2. Whereas, TPC had significant effect with all linear and quadratic terms. The quadratic analysis was confirmed by the statistical importance of quadratic terms ($p < 0.05$) [18] Table 1.

$$\begin{aligned} \text{TPC} = & 765.76 - 5.64X_1 - 8.57X_2 - 7.33 X_3 - 2.08X_4 + 1.25 X_1X_2 + 7.76X_1X_3 + 0.12X_1X_4 + \\ & 3.78X_2X_3 + 0.25X_2X_4 + 2.96 X_3X_4 - 68.98X_1^2 - 13.11 X_2^2 - 14.20 X_3^2 - 6.61X_4^2 \end{aligned} \text{ Eq. 16}$$

The high R^2 (0.93) and adjusted R^2 (0.86) values indicate high predictive ability, CV value of 1.78% indicates low relative standard deviation and F-value, 14.00, indicated the statistical significance of the model. Furthermore, for TPC model adequate precision of 10.326 indicated

392 strong signals, confirming the models' suitability for design space navigation. US in
393 combination with enzymatic maceration was found effective for the enhancement of bioactive
394 compounds from jamun pulp. Sonication first disrupted the fruit tissue and softened the cell
395 wall structures, thus enhancing the accessibility of enzyme macromolecules to
396 their respective substrates. The enhanced retention of phenolic compounds is essentially as a
397 result of the transformation of bound, insoluble phenolics into free, soluble
398 forms due to the treatment using ultrasound. Studies also suggest that ultrasound has a direct
399 influence on the internal enzymatic activity of fruits, thereby producing reactive oxygen
400 species and aiding in the process of phenolic extraction [18]. Moreover, the interaction of
401 sonication time and amplitude increase TPC (Fig. 2) lead to the formation of hydroxyl radicals,
402 where the radicals might have facilitated hydroxylation of phenolic compounds at para, meta,
403 and ortho positions of the aromatic ring structure.
404 Nevertheless, prolonged sonication adversely affects the overall phenolic content (TPC),
405 as suggested by the quadratic term X4. The continued generation of free radicals throughout
406 cavitation may also result in the degradation of TPC. On the other hand, the low concentration
407 of phenol in control sample might be due to oxidative degradation of thermally sensitive
408 phenolic compounds. [42, 43, 10, 39]. In addition, the polyphenol oxidase inactivation and
409 elimination of dissolved oxygen during processing also contribute to the increased retention
410 rate [11]. At the same time when sonication time increased (X_1) phenolic compounds reacted
411 with sulfhydryl and amine groups in proteins, resulting in the formation of irreversible and
412 insoluble complexes and the entire process accounts for the lower phenolic content in clarified
413 pulp [12]. Similar study [44] found that the polyphenolic contents in apple juice increased
414 remarkably at significance level $p \leq 0.05$ after being treated with ultrasound at 20°C. This
415 increase is expected to be due to the sudden pressure fluctuations and shear forces resulting
416 from collapsing bubbles, creating cavitation and breaking down the cell structure.

An increase in TPC after enzymatic treatment of the pulp may be due to the breakdown of cell wall by the enzymes, allowing the free and bound phenolic compounds to be released into the pulp. Fig. 2 Response surface plots were employed to verify the impact of interaction among process variables on TPC [21].

3.1.2 Impact of UAEZ on Acn composition of jamun pulp

The Acn content of treated sample ranges from 35.48 mg/100g to 38.84 mg/100g. Similar to TPC model interaction and quadratic terms were identified as significant ($p < 0.05$) and the interaction terms exhibited a positive effect on the model, whereas the interaction of X_2X_3 and X_3X_4 had a negative impact on the Acn composition as illustrated in Fig. 3 [18]. Additionally, independent variables had significant negative impact on the linear and quadratic effect on Acn yield. The regression equation obtained for total Acn content is given below:

$$\text{Acn} = 38.57 - 0.33X_1 - 0.35X_2 - 0.33X_3 - 0.053X_4 + 0.11 X_1X_2 + 0.14X_1X_3 + 0.037X_1X_4 - 0.037X_2X_3 + 0.03X_2X_4 - 0.012X_3X_4 - 2.12X_1^2 - 0.43X_2^2 - 0.42X_3^2 - 0.067X_4^2 \quad \text{Eq. 17}$$

The R^2 , adjusted R^2 , CV, F value and adequate precision of the model were 0.92, 0.84, 1.18%, 12.29 and 10.35 indicates high predictive ability, statistically significance and strong signals, confirming the models' suitability for design space navigation. The interaction between ultrasonication time and enzyme concentration increased (Fig. 3) penetration of water to fruit cell wall through turbulence and microstreaming and this in turn improves the infiltration of cellular structure which ease the diffusion of bioactive compounds to the surrounding medium i.e. solvent [21, 16]. It was also stated that ultrasound treatments do not break down Acn directly. However, at more elevated intensities, i.e. with increased sonication time and amplitude ultrasound causes cell walls to become severely disrupted, allowing easy efflux of Acn into the pulp. In addition, at high intense power of 200 W sonication may effectively inhibited the activities of peroxidase and polyphenol oxidase, the two crucial enzymes involved

in Acn degradation and pulp browning [39]. Pectinase enzyme also helps in degradation of pectin in the cell wall and released bound form of Acn present in jamun. This happened due to appropriate solid (jamun pulp) to liquid (water) ratio and when the ratio was increase in preliminary trials hydrolysis of glycosidic bond of Acn increased in the later phase which leads to structural disintegration, and decreased Acn content [45]. Enzymatic treatment at optimal incubation temperature (45°) also promoted enzymatic hydrolysis and gradually release Acn concentration. However, when the temperature exceeds 45°C, anthocyanin content decreases, probably because of the reduced enzyme activity in hydrolysis process [45]. In case of control samples rise in temperature facilitated the softening of plant tissues and altered the physical properties of the solvent, enhancing mass transfer rates and improving Acn solubility [46]. But elevation in temperatures increase the formation of chalcone, which continues until their complete degradation.

3.1.3 Impact of USEZT on DPPH activity

Jamuns are widely recognized for their strong antioxidant properties because of the combined effects of several bioactive compounds, including polysaccharides, flavonoids, phenolic acids, and ascorbic acid [10, 47]. The USEZT exhibited a significant increase in TPC, which might be due to increased levels of Acn in the pulp. Acn, along with their glycosidic derivatives, also have significant antioxidant activity [48]. Fig. 4 depicts response surface plots employed to verify the impact of interaction among process variables on DPPH activity [21]. The regression equation for DPPH scavenging activity is as follows:

$$\text{DPPH activity} = 69.41 - 0.38X_1 - 1.11X_2 - 1.20 X_3 - 0.14X_4 + 0.02 X_1X_2 + 0.17X_1X_3 + 0.01X_1X_4 + 0.48X_2X_3 + 0.04X_2X_4 + 0.03 X_3X_4 - 6.17X_1^2 - 2.01 X_2^2 - 2.11 X_3^2 - 1.21X_4^2 \text{ Eq. 18}$$

The model was statistically significant ($p < 0.05$), with R^2 and adjusted R^2 of 0.85 and 0.70, respectively, showing a high correlation between the predicted and actual values. Moreover,

an acceptable precision value of 7.44 suggests that the model is safe for investigating the design space. The DPPH scavenging activity varied from 60.13 to 69.99% across the model and the enhanced free radical scavenging activity in pulp samples is more or less influenced by ascorbic acid and phenolic compounds. Moreover, cavitation during sonication leads to the collapse of surrounding colloidal particles, and facilitating better release of TPC from the cell wall [43, 49]. Phenols, being redox active they can function as a reducing agent, hydrogen donor, and metal chelator [44]. When sonication time increased moderately, more antioxidants were released into fruit extracts, which helped to scavenge more free radicals [42]. In the study maximum peak for antioxidant activity was achieved at a peak up to sonication time of 30 min. However, ultrasonication applied at high intensity generated excessive reactive oxygen species which in turn reduced the antioxidant property of the fruit and oxidized the phenolic compounds present in pulp [30]. It was observed that with a sonication time duration of 30 to 60 min (X_4), antioxidant activity decreased because cavitation bubbles either weakened or grew too much, failing to collapse, and hence reducing their impacts. Moreover, the uncontrolled presence of too many cavitation bubbles might hinder ultrasonic wave propagation [50]. In the next stage of enzymatic maceration aggregation of pectin complex after hydrolysis process, effect the cell-wall structure and reducing its water retention ability aiding in better extraction of phenolic compounds, such as Acn which in turn increased antioxidant activity [48] and it was observed at preliminary trails the incubation above 45°C reduced DPPH activity because at elevated temperature antioxidants readily evaporates at a given high volatility, which suffers significant losses. In control samples extended duration of exposure at high temperatures results in a great loss of antioxidant activity.

Using RSM model, the best USEZT treatment conditions was determined as follows: X_1 - Ultrasound time: 25 minutes, X_2 - Amplitude: 20%, X_3 - Enzyme concentration: 0.45, and X_4 - Incubation time: 60 minutes. The bioactive composition values obtained under these

optimized conditions, as predicted by the design model and confirmed through experiments, were: TPC: 768 mg/100g and 760 ± 0.03 mg/100g, Acn content: 38.71 mg/100g and 38.57 ± 0.10 mg/100g, DPPH scavenging activity: 69.77% and $69.63 \pm 0.08\%$. The minor variation between the predicted and experimental values might be due to environmental factors. Furthermore, both control and RSM-optimized treatment conditions were subject to a physicochemical analysis (Table 3).

3.2 Physicochemical analysis of control and optimized pulp samples

3.2.1 Moisture content, juice yield and viscosity

Optimized USEZT treated pulp had significantly more moisture ($95.43 \pm 0.12\%$) than the control ($92.12 \pm 0.62\%$) sample and this might be due to the cleavage of pectin glycosidic bond by hydrolysis, which promoted the penetration of water into the oxygen bridge and trans elimination lysis owing to pectin degradation mechanisms [24]. Cavitation action also played a role in breaking down the fruit cell walls, erosion of the surface, and fragmentation of some particles, which helped to improve mass transfer and juice release [16]. Jamun pulp extraction is primarily dependent on the release of water-soluble pectin derived from protopectin [51]. In a similar study for Sohiong pulp [21], a significant rise in juice yield following hydrolysis of protopectin by pectinase enzyme led to the breakdown and release of water-soluble pectin and solubilization of insoluble cell wall materials such as pectin, hemicelluloses, and some cellulose materials favourable for better release of juice from the structural network [24]. In the current study ultrasonication followed by pectinase enzyme treatment efficiently hydrolyses glycosidic bonds in polygalacturonic acid i.e. i.e. cleavage of the α -1,4 glycosidic bond and hydrolysis of the methoxy groups of the pectin structure, degrading it to monogalacturonic acid, enhancing juice recovery from jamun juice. Juice yield also increased with appropriate incubation temperature up to 40°C . But in preliminary trials juice extraction

efficiency decreases thereafter, possibly because the enzyme is less efficient at higher temperatures (50 to 60°C). The juice yield increases linearly with incubation time and this is most likely because the enzyme has more time to act on the fruit pulp [23]. Pectinase also has a greater affinity in lowering the viscosity of jamun pulp as compared to control juice sample due to the high-water holding water capacity and ability to develop a cohesive networked structure. When pectin breaks down enzymatically, its capacity to hold water is reduced and hence free water is released in the juice system and viscosity decreases [26]. In a similar study enzyme-treated carrot juice samples showed slightly lower viscosity than the untreated sample, and this is expected due to pectolytic enzymes breaking the carrot cell wall, which is rich in protopectin and pectin [36].

3.2.2 pH, titratable acidity and ascorbic acid

USEZT samples slightly reduced the pH from 3.46 ± 0.05 to 3.20 ± 0.02 in comparison to the control sample (Table 3). The drop in pH indicates the USEZT sample to be more acidic. The change in pH was associated with heat stress, which modifies certain of its physicochemical characteristics [12]. The major tautomer at very low pH is the red-coloured flavylum cation. Proton loss occurs relatively fast with increasing pH and develops to purple and blue quinonoid species. This is attributed to the pH-dependent structural transformation of Acn [49]. Titratable acidity of control sample and USEZT extracted jamun pulp was $0.54 \pm 0.01\%$ and $1.38 \pm 0.05\%$, respectively. The rise in titratable acidity could be because of the activity of pectinase towards cell wall materials, i.e., protopectin and pectin, resulting in higher acidic content in the end [24, 12]. The rise in acidity was due to the drop in pH, [52] where pectinase treatment might have released carboxylic and galacturonic acid monomers, which are responsible for additional increased acidity in the extracted pulp [12]. Variations in acidity can be caused by ion fermentation during sonolysis of water or bioactive agents, or by the creation of byproducts created through oxidative degradation [53]. An increase in

acidity can also hinders microbe growth and therefore extends pulp shelf life [52]. The fall in pH in control sample may be due to de-esterification of heavily methylated pectin molecules [24].

Raw pulp and USEZT had 22.69 ± 0.42 mg/100g and 24.91 ± 0.40 mg/100g ascorbic acid content, respectively (Table 3). Sonication process effectively removed dissolved oxygen during the treatment time of 30°C for 25 minutes, which in turn enhanced ascorbic acid stability during cavitation process [43, 30]. Whereas the longer sonication time beyond 25 minutes lead to intense sonolytic activity which cause bubble collapse during cavitation and helped in the production of free radicals and lead to the degradation of ascorbic acid [19, 30]. Moreover, it was stated [43] that ascorbic acid degradation is associated with oxidative reactions under aerobic and anaerobic conditions and with the formation and scavenging of hydroxyl radicals. Further the inactivation of ascorbic acid oxidase increased the retention rate of ascorbic acid in treated pulp samples. Thermal treatment also preserves more ascorbic acid, although it is partially lost during heat treatment [54]. But the drastic reduction in the quantity of ascorbic acid in thermally processed pulp may be due to its sensitivity to temperature, as increased heat speeds up its degradation process [39].

3.2.3 Total soluble solids and total sugar content

At the optimum process parameters like ultrasound treatment time and amplitude hydrolysed the glycosidic bonds i.e. pectic compounds and released the dissolved compounds present in pulp [21]. In addition, in the second extraction stage, total soluble solids (TSS) further rose as pectinase destroyed the tissue structure, releasing more soluble substance into the pulp and promoting the release of sugars [48, 24, 16]. A similar study by [12] explained that TSS of pear juice obtained from enzymatic extraction were attributed to the depolymerization of polygalacturonase chains by pectinases resulting in the liberation of

reducing sugars, in addition to the hydrolysis of non-reducing sugars under acid catalysis at elevated temperature [24]. TSS of treated sample might have also increased due to the better release of sugars (fructose and sucrose) present in jamun pulp. In the study TSS ($9 \pm 0.11\%$) content of optimized pulp sample was more in comparison to control ($8.10 \pm 1.15\%$) (Table 3), which might be attributed to the hydrolysis of insoluble pectin or due to breakdown of cell wall polysaccharides after USEZT, which enabled the better release of sugars from the tissues of the jamun pulp [12, 16]. Similarly, the high reducing sugar content in USEZT samples ($6.67 \pm 0.11\%$) was attributed to the mechanical action of US which enhanced extraction efficiency, liberating entrapped sugar particles into the pulp [Manzoor et al., 2020]. Reduction in sugar content in control samples can be ascribed to the thermal degradation of sugars (glucose and fructose) which was most likely due to the thermal pasteurization [39].

3.2.4 Protein and crude fibre content

In ultrasonic-assisted extraction, ultrasonic waves promote mass transfer, which causes the release of polyphenols and proteins from the parenchyma cell wall by the cavitation effect [55]. The crude protein content of the control (0.37 ± 0.05 g/100 mL) was more in comparison to USEZT sample (0.34 ± 0.10 g/100mL). Comparable research was documented for pear juice where protein content of pear juice extracted enzymatically ($0.33 \pm 0.01\%$) was considerably lower than the control ($0.41 \pm 0.02\%$) sample [12,54]. The loss in protein content was due to ultrasonic cavitation, which broke peptide and hydrogen bonds of the protein, leading to changes in its secondary and tertiary structures. Such structural changes indicate that ultrasound is capable of altering protein conformations or cleaving polypeptide chains and, as such, may impact the allergenicity of certain food proteins [56]. The cavitation-induced high shear forces and microstreaming phenomena may break van der Waals forces and hydrogen bonding, contributing to protein denaturation [57]. Further, enzymatic pectin breakdown, mainly by the action of pectinases, indirectly affects the stability of protein [26].

Pectinase hydrolysed pectin chains under favourable incubation conditions and exposes positively charged proteins. Electrostatic interaction between pectin and the protein causes particle agglomeration, which causes sedimentation and decreased protein content [24]. Additionally, enzymatic extraction caused loss of dietary fibre due to the breakdown and solubilization of insoluble components such as cellulose and hemicellulose. Treated pulp also had lower dietary fibre (0.87 ± 0.17 g/100 mL) content in comparison to the control sample (1.10 ± 0.11 g/100 mL).

3.2.5 Colour value and colour density

Relative to the control, redness (a^*) increased from 26.22 ± 0.01 to 31.29 ± 0.02 under optimal USEZT conditions, whereas lightness (L^*) and yellowness (b^*) decreased from 8.54 ± 0.17 to 6.17 ± 0.02 and -6.91 ± 0.02 to -7.66 ± 0.15 , respectively (Table 3). The rise in a^* value might be due to partial settling of unstable suspended particles during cavitation process and this process tends to favor the creation of more coloured molecules in pulp [30]. Enzyme activity was also responsible for tissue structural degradation, releasing colour-bearing constituents responsible for reduced lightness (L^*) of samples. Whereas the control sample treated with thermal pasteurization leads to much dark coloured pulp samples due to the hydroxylation of the aromatic ring of phenolic compounds might have led to darkening the red coloration and decreased a^* value significantly [39]. Total colour difference, ΔE 5.64 indicates a distinctly noticeable to moderate colour change between treated and control Jamun pulp samples, which would mean the treatment significantly changed the colour of the pulp that might have affected consumer perception, illustrating that the USEZT resulted in colour alterations in jamun pulp. Colour density is a universal marker for testing Acn polymerization and browning, which was induced by the degradation of monomeric Acn in fruit pulp. But in the study USEZT samples

(Table 3) slightly enhanced CD value due to the enhanced dissolution of Acn due to sonication treatment, leading to higher absorbance in the visible region at 420 nm and 510 nm [12, 39].

3.2.6 Sensory attributes

It was observed that control and JP1 (USEZT) samples had apparently comparable sensory rating to that of control sample. In sensory profile flavour and colour were considered as an important attribute to attract consumers and in the current study there was only slight variation in the flavour scores between control (7.1) and treated pulp samples JP1 (7.2) and JP2 (7.2). Whereas colour score of control sample (7.8) had slightly highest rating than JP1 (7.5) indicating the positive correlation of treatment conditions on the visual characteristic of test samples. Similarly, odour and texture rating of JPI (7.3 and 7.4) sample was much preferred over the control sample (6.9 and 6.8). But the samples JP3 and JP4 received the least sensory score among all the sensory attributes particularly for flavour (6 and 5.6) and odour (6.6, 5.8) which indicated the samples to be least preferred. The high concentration of TPC resulted in USEZT pulp samples with more bitter, sour and astringent, bitter and sour sensory characteristics.

3.2.7 HPLC profiling

Ferulic acid and quercetin had maximum absorbance at 320 nm whereas gallic acid had maximum absorbance at wavelength of 280 nm. Among the three phenolic acids identified, the most dominant compound was gallic acid, with maximum concentration of 42.10 ± 0.55 mg/100g whereas control sample had gallic acid concentration of 37.39 ± 0.52 mg/100g (Table 3). In the same manner, ferulic acid (0.91 ± 0.08 mg/100g) was found to be the second most abundant phenolic compound in USEZT pulp sample and least phenolic concentration of 0.48 ± 0.02 mg/100g and 0.80 ± 0.05 mg/100g of quercetin was detected in control and treated sample.

Maximum absorbance of ferulic acid and quercetin was because of their extended conjugation, whereas gallic acid absorbance at 280 nm owns to its less complex aromatic structure [58]. These phenolics are a major source of the antioxidant activity in jamun, with gallic acid being the highest active radical-scavenging agent. Ferulic acid and quercetin, although found in lower levels, also increased overall antioxidant potential because of their synergistic actions [59].

3.2.8 Jamun pulp particle size distribution (PSD)

The particle size of jamun pulp was determined by dynamic light scattering based on the Brownian motion of suspended particles resulting from collisions with solvent molecules. It was also observed that freezing and thawing of pulp increased the particle size and resulted in a wider particle size distribution. Particle size variations also affect the sedimentation behaviour and more evenly distributed particle size improves pulp stability. In the study USEZT treatments resulted in better PSD in comparison to control sample. Enzymatic treatment affected the size of particles in jamun pulp, whereas cavitation energy produced shock waves that increased local pressure, causing cellulose particles to break down. Surface erosion and cellulose fragment formation, lead to a decrease in particle size, result from the process [43].

The PSD of control sample as shown in Fig. 5a, exhibit a broader distribution with multiple peaks (100 and 1000 nm), suggesting a more heterogeneous particle size range which indicates particle aggregation or the presence of different particle fractions, which could impact stability. Whereas USEZT profoundly decreased the particle size of pulp, evident from a more defined single peak at 200nm, implying a relatively uniform particle population [16] and the suitability of solid particles present in pulp in new product formulations, nutraceuticals and pharmaceutical sectors. USEZT pulp also had much lower polydispersity (0.43) as compared

to control sample (0.26). A polydispersity index less than 0.5 signifies that a relatively narrow particle size distribution is present, which is critical to ensure consistency. The treated sample also had a zeta potential value of -17mV . Zeta potential between -15 mV and -30 mV validates the stability of the solid particles and ensure minimal aggregation of solid particles [41,60].

Conclusion

The current research proved the efficacy of USEZT for improving the content of bioactive composition of jamun pulp. Decrease in particle size and polydispersity index further reflects the possibility of treated sample to form more stable pulp formulations for multiple applications such as nutraceuticals and pharmaceuticals. Moreover, the elevation of beneficial phenolic compounds like gallic acid, ferulic acid, and quercetin attribute towards the antioxidant activity of jamun pulp. Although the study ensures identification of the effect of USEZT on jamun pulp samples, more research focus should be to assess the long-term stability of Acn and phenolic compounds during storage. Also, the present research mainly covers physicochemical and sensory characteristics; the subsequent studies should also estimate the microbiological and long-term stability of bioactive compounds present treated pulp and thereby evaluate the commercial potential. Overall, the current study provides an insight about the viability of USEZT as a green and efficient technique for improving the functional properties of jamun pulp. Addressing these knowledge gaps will ensure further streamlining of the technology and its acceptance in the food and beverage sector.

Acknowledgments

The authors gratefully acknowledge the Department of Food Science and Technology, Lovely Professional University, Punjab, India, for providing the necessary support and facilities that helped in completing this research paper successfully.

683 **Authors contribution**

684 B. S. Adithya: Writing original draft, investigation; formal analysis; visualization;. Sourabh
685 Kumar: Methodology; visualization; supervision. Swarup Roy: Methodology; project
686 administration, conceptualization; visualization; supervision; resources, review and editing.
687 Ravindra Kumar Tiwari: Methodology; visualization; supervision and editing. Sagar, N.A.:
688 Resources and review and editing.

689 **Compliance with ethical standards**

690 Conflict of interest and ethical issues: The author doesn't have any conflict of interests and
691 ethical issues to declare.

692 **Funding**

693 The research did not receive any specific grant from funding agencies in the commercial, public
694 or not-for-profit sectors.

695 **Data Availability**

696 Data is provided within the manuscript or supplementary information files.

697 **Declaration of Interest**

698 The authors declare that they have no known competing financial interests or personal
699 relationships that could have appeared to influence the work reported in this paper.

700 **Clinical trial number**

701 Not applicable

702 **Human Ethics and Consent to Participate declarations**

703 The sensory analysis protocol was approved by National Accreditation Board for Testing &
704 Calibration Laboratories (NABL) certified agency [Fare Labs Pvt. Ltd.: ISO/IEC 17025:2017]

in accordance with the [Food Safety Standards Authority of India (FSSAI), India]. As per the consent letter (J.O. No. FL/FA/20042024-138), informed consent of panelists was obtained to participate in the study.

Consent to Publish declaration

All authors have mutual consent to publish the manuscript.

Collection of the plant material

All local and national Indian norms and regulations were followed while collecting the ‘Ra’ variety of Jamun fruits used in this research work. The fruits were collected ethically without endangering the native population or ecology, and no protected or endangered species were involved. The fruit was also classified as a species of Least Concern in 2018 by the International Union for Conservation of Nature in Red List of Threatened Species review.

References

1. S. Ahmed, I.A. Jahan, M.H. Hossain, K.S. Ahmed, M. Rahman, W. Zaman, M.M. Hoque, Bioactive compounds, antioxidant properties and phenolic profile of pulp and seed of *Syzygium cumini*. J. Food Meas. Charact. 15(2) (2021).
<https://doi.org/10.1007/s11694-020-00798-2>
2. E.M. Coelho, L.C. De Azevedo, L.C. Corrêa, M.T. Bordignon-Luiz, M.D.S. Lima, Phenolic profile, organic acids and antioxidant activity of frozen pulp and juice of the jambolan (*Syzygium cumini*). J. Food Biochem. 40(2) (2016).
<https://doi.org/10.1111/jfbc.12209>
3. S. Ding, H. Jiang, J. Fang, Regulation of immune function by polyphenols. J. Immunol. Res. (2018). <https://doi.org/10.1155/2018/1264074>

4. F. Han, P. Yang, H. Wang, I. Fernandes, N. Mateus, Y. Liu, Digestion and absorption of red grape and wine anthocyanins through the gastrointestinal tract. Trends Food Sci. Technol. 83 (2019). <https://doi.org/10.1016/j.tifs.2018.11.025>
5. J.P. Singh, A. Kaur, N. Singh, L. Nim, K. Shevkani, H. Kaur, D.S. Arora, In vitro antioxidant and antimicrobial properties of jambolan (*Syzygium cumini*) fruit polyphenols. LWT. 65 (2016). <https://doi.org/10.1016/j.lwt.2015.09.038>
6. J. Xu, T. Liu, Y. Li, W. Liu, Z. Ding, H. Ma, N.P. Seeram, Y. Mu, X. Huang, L. Li, Jamun (*Eugenia jambolana* Lam.) fruit extract prevents obesity by modulating the gut microbiome in high-fat-diet-fed mice, Mol. Nutr. Food Res. 63(9) (2019). <https://doi.org/10.1002/mnfr.201801307>
7. P. Ghosh, N. Swaraj, R.C. Pradhan, Effect of tannase (*Aspergillus ficcum*) on physicochemical properties of clarified Jamun juice. Agric. Eng. Int. CIGR J. 23(1) (2021). <http://orcid.org/0000-0002-6872-6057>
8. I.D. Paul, M. Das, Effect of freeze, microwave-convective hot air, vacuum and dehumidified air drying on total phenolics content, anthocyanin content and antioxidant activity of jamun (*Syzygium cumini* L.) pulp. J. Food Sci. Technol. 55 (2018). <https://doi.org/10.1007/s13197-018-3158-2>
9. U.M. Ogbeide, D.E. Irene, H.A. Okeri, Assessment of pH, titratable acidity, and caffeine content of some brands of energy drinks. J. Sci. Pract. Pharm. 8(1) (2021). <https://doi.org/10.47227/jsppharm.v8i1.4>
10. D. Pradhan, H. Baishya, A.B. Das, Effect of ultrasound, centrifugation, and enzymatic pretreatments on phytochemical properties and membrane fouling during microfiltration of Sohiong (*Prunus nepalensis* L.) juice. Food Sci. Biotechnol. 31(11) (2022). <https://doi.org/10.1007/s10068-022-01129-5>

11. Wang, Q. Liu, B. Xie, Z. Sun, Effect of ultrasound combined with ultraviolet treatment on microbial inactivation and quality properties of mango juice. *Ultrasonics Sonochemistry*. 64 (2020). <https://doi.org/10.1016/j.ultsonch.2020.105000>
12. G. Gani, H.R. Naik, N. Jan, O. Bashir, S.Z. Hussain, A.H. Rather, M. Reshi, T. Amin, Physicochemical and antioxidant properties of pear juice prepared through pectinase enzyme-assisted extraction from William Bartlett variety. *J. Food Meas. Charact.* 15 (2021). <https://doi.org/10.1007/s11694-020-00676-x>
13. P. Ghosh, R.C. 41, S. Mishra, Jamun juice extraction using commercial enzymes and optimization of the treatment with the help of physicochemical, nutritional and sensory properties. *Int. J. Nutr. Food Eng.* 11(1) (2017). <https://doi.org/10.5281/zenodo.1339890>
14. A. Sonawane, S.S. Pathak, R.C. Pradhan, Optimization of a process for the enzymatic extraction of nutrient enriched bael fruit juice using artificial neural network (ANN) and response surface methodology (RSM). *Int. J. Fruit Sci.* 20(sup3) (2020). <https://doi.org/10.1080/15538362.2020.1834898>
15. A. Marsol-Vall, N. Kelanne, A. Nuutinen, B. Yang, O. Laaksonen, Influence of enzymatic treatment on the chemical composition of lingonberry (*Vaccinium vitis-idaea*) juice. *Food Chem.* 339 (2021). <https://doi.org/10.1016/j.foodchem.2020.128052>
16. S. Wang, Z. Liu, S. Zhao, L. Zhang, C. Li, S. Liu, Effect of combined ultrasonic and enzymatic extraction technique on the quality of noni (*Morinda citrifolia* L.) juice. *Ultrasonics Sonochemistry*. 92 (2023). <https://doi.org/10.1016/j.ultsonch.2022.106231>
17. J. Luo, Z. Fan, X. Yang, Y.H. Bao, M. Liang, Y. Guo, Anthocyanins and antioxidant activity of *Lonicera caerulea* berry wine during different processes. *Food Sci. Technol.* 42 (2021). <https://doi.org/10.1590/fst.25121>

18. T.T. Le, N.L. Le, Antioxidant capacities and betacyanin LC-MS profile of red-fleshed dragon fruit juice (*Hylocereus polyrhizus*) extracted by ultrasound-assisted enzymatic treatment and optimized by response surface methodology. J. Food Process. Preserv. 45(3) (2021). <https://doi.org/10.1111/jfpp.15217>
19. M. Dadan, A. Grobelna, S. Kalisz, D. Witrowa-Rajchert, The impact of ultrasound-assisted thawing on the bioactive components in juices obtained from blue honeysuckle (*Lonicera caerulea* L.). Ultrason. Sonochem. 89 (2022). <https://doi.org/10.1016/j.ultsonch.2022.106156>
20. S.C. Bavisetty, K. Venkatachalam, Physicochemical qualities and antioxidant properties of juice extracted from ripe and overripe wax apple as affected by pasteurization and sonication. J. Food Process. Preserv. 45(6) (2021). <https://doi.org/10.1111/jfpp.15524>
21. K. Vivek, S. Mishra, R.C. Pradhan, Optimization of ultrasound-assisted enzymatic extraction of Sohiong (*Prunus nepalensis*) juice. J. Food Process Eng. 42(1) (2019). <https://doi.org/10.1111/jfpe.12948>
22. D. Pradhan, S. Abdullah, R.C. Pradhan, Optimization of pectinase assisted extraction of Chironji (*Buchanania lanzan*) fruit juice using response surface methodology and artificial neural network. Int. J. Fruit Sci. 20(sup2) (2020). <https://doi.org/10.1080/15538362.2020.1734895>
23. P. Ghosh, R.C. Pradhan, S. Mishra, Optimization of process parameters for enhanced production of Jamun juice using Pectinase (*Aspergillus aculeatus*) enzyme and its characterization. 3 Biotech. 6(2) (2016). <https://doi.org/10.1007/s13205-016-0561-0>
24. O. Bashir, S.Z. Hussain, G. Gani, N. Jan, A.H. Rather, M. Reshi, T. Amin, Evaluating the physicochemical and antioxidant characteristics of apricot juice prepared through

pectinase enzyme-assisted extraction from Halman variety. J. Food Meas. Charact. 15 (2021). <https://doi.org/10.1007/s11694-021-00833-w>

25. M.M. Hasan, P. Roy, M. Alam, M.M. Hoque, W. Zzaman, Antimicrobial activity of peels and physicochemical properties of juice prepared from indigenous citrus fruits of Sylhet region, Bangladesh. Heliyon. 8(7) (2022). <https://doi.org/10.1016/j.heliyon.2022.e09948>

26. P. Arsad, R. Sukor, W.Z. Wan Ibadullah, N.A. Mustapha, A.S. Meor Hussin, Effects of enzymatic treatment on physicochemical properties of sugar palm fruit juice. Int. J. Adv. Sci. Eng. Inf. Technol. 5(5) (2015).

27. J.H. Kim, J.H. Kim, J.B. Eun, Optimization of spray drying process of Japanese apricot (*Prunus mume* Sieb. et Zucc.) juice powder using nondigestible maltodextrin by response surface methodology (RSM). J. Food Sci. Technol. 60(3) (2023). <https://doi.org/10.1007/s13197-020-04917-1>

28. H. Twinomuhwezi, C.G. Awuchi, M. Rachael, Comparative study of the proximate composition and functional properties of composite flours of amaranth, rice, millet, and soybean, Am. J. Food Sci. Nutr. 6(1) (2020). <http://www.aascit.org/journal/ajfsn>

29. A.A. de Brito, F. Campos, A. dos Reis Nascimento, C. Damiani, F.A. da Silva, G.H. de Almeida Teixeira, L.C. Júnior, Non-destructive determination of color, titratable acidity, and dry matter in intact tomatoes using a portable Vis-NIR spectrometer. J. Food Compos. Anal. 107 (2022). <https://doi.org/10.1016/j.jfca.2021.104288>

30. A.O. Oladunjoye, F.O. Adeboyejo, T.A. Okekunbi, O.R. Aderibigbe, Effect of thermosonication on quality attributes of hog plum (*Spondias mombin* L.) juice. Ultrasonic Sonochemistry. 70 (2021). <https://doi.org/10.1016/j.ultsonch.2020.105316>

31. S. Kaur, N. Kaur, P. Aggarwal, K. Grover, Bioactive compounds, antioxidant activity, and color retention of beetroot (*Beta vulgaris* L.) powder: Effect of steam blanching

824 with refrigeration and storage. J. Food Process. Preserv. 45(3) (2021).
825 <https://doi.org/10.1111/jfpp.15247>

826 32. P.K. Dubey, K.S. Kumar, A. Rawson, S. Anandakumar, N. Baskaran, S. Thamburaj,
827 Effect of pulsed electric field on physicochemical properties of rice and black gram
828 fermented batter. J. Food Process Eng. 46(9) (2023). <https://doi.org/10.1111/jfpe.14411>

829 33. N. George, A.A. Andersson, R. Andersson, A. Kamal-Eldin, Lignin is the main
830 determinant of total dietary fiber differences between date fruit (*Phoenix dactylifera* L.)
831 varieties. NFS J. 21 (2020). <https://doi.org/10.1016/j.nfs.2020.08.002>

832 34. Food Safety and Standards Authority of India, Manual of Methods of Analysis of Food.
833 Fruit and Vegetable Products New Delhi.(2012).
834 <http://fssai.gov.in/Portals/0/Pdf/15Manuals/FRUITS%20AND%20VEGETABLES.pdf>
835 [f](http://fssai.gov.in/Portals/0/Pdf/15Manuals/FRUITS%20AND%20VEGETABLES.pdf)

836 35. S. Kapoor, P.S. Ranote, Antioxidant components and physico-chemical characteristics
837 of jamun powder supplemented pear juice. J. Food Sci. Technol. 53 (2016).
838 <https://doi.org/10.1007/s13197-016-2196-x>

839 36. M. Kaur, H.K. Sharma, Effect of enzymatic treatment on carrot cell wall for increased
840 juice yield and effect on physicochemical parameters. Afr. J. Plant Sci. 7(6) (2013).
841 <http://www.academicjournals.org/AJPS>

842 37. S.D. Maduwanthi, R.A. Marapana, Total phenolics, flavonoids and antioxidant activity
843 following simulated gastro-intestinal digestion and dialysis of banana (*Musa*
844 *acuminata*, AAB) as affected by induced ripening agents. Food Chem. 339 (2021).
845 <https://doi.org/10.1016/j.foodchem.2020.127909>

38. N. Chaves, A. Santiago, J.C. Alías, Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. *Antioxidants*. 9(1) (2020). <https://doi.org/10.3390/antiox9010076>
39. W. Wu, G. Xiao, Y. Yu, Y. Xu, J. Wu, J. Peng, L. Li, Effects of high pressure and thermal processing on quality properties and volatile compounds of pineapple fruit juice. *Food Control*. 130 (2021). <https://doi.org/10.1016/j.foodcont.2021.108293>
40. N. Kaur, P. Aggarwal, V. Kumar, S. Kaur, Ultrasound-assisted extraction of phytochemicals from java plum (*Syzygium cumini* L.) pomace: Process optimization, phytochemical characterization using HPLC, FTIR, SEM and mineral profiling. *Waste Biomass Valor*. 14(3) (2023). <https://doi.org/10.1007/s12649-022-01915-6>
41. U. Akbar, P. Rasane, M.S. Mondol, J. Singh, V. Nanda, H. KILIÇGÜN, S. Kaur, Impact of particle size on physical and functional characteristics of baby corn powder. *Turk. J. Agric. For*. 49(1) (2025). <https://doi.org/10.55730/1300-011X.3250>
42. R.M. Aadil, A.A. Khalil, A. Rehman, A. Khalid, M. Inam-ur-Raheem, A. Karim, A.A. Gill, M. Abid, M.T. Afraz, Assessing the impact of ultra-sonication and thermo-ultrasound on antioxidant indices and polyphenolic profile of apple-grape juice blend. *J. Food Process. Preserv*. 44(5) (2020). <https://doi.org/10.1111/jfpp.14406>
43. M.F. Manzoor, M. Ali, R.M. Aadil, A. Ali, G. Goksen, J. Li, X.A. Zeng, C. Proestos, Sustainable emerging sonication processing: Impact on fungicide reduction and the overall quality characteristics of tomato juice. *Ultrasonics Sonochemistry*. 94 (2023). <https://doi.org/10.1016/j.ultsonch.2023.106313>
44. M. Nadeem, M.M. Ranjha, K. Ameer, A. Ainee, Z. Yasmin, S. Javaria, T.F. Teferra, Effect of sonication on the functional properties of different citrus fruit juices. *Int. J. Fruit Sci*. 22(1) (2022). <https://doi.org/10.1080/15538362.2022.2079584>

45. Y. Li, F. Tao, Y. Wang, K. Cui, J. Cao, C. Cui, L. Nan, Y. Li, J. Yang, Z. Wang, Process optimization for enzymatic assisted extraction of anthocyanins from the mulberry wine residue. IOP Conf. Ser. Earth Environ. Sci. 559(1) (2020). <https://doi.org/10.1088/1755-1315/559/1/012011>
46. M. de Moraes Sousa, R.M. de Lima, A. de Lima, A.C. Reis, A.A. Cavalcante, J.A. Sattler, L.B. de Almeida-Muradian, J.D. Neto, R.S. dos Reis Moreira-Araujo, N. do Nascimento Nogueira, Antioxidant action and enzyme activity modulation by bioaccessible polyphenols from jambolan (*Syzygium cumini* (L.) Skeels). Food Chem. 363 (2021). <https://doi.org/10.1016/j.foodchem.2021.130353>
47. X. Shi, X. Wang, Y. Zheng, L. Fu, Advances in the study of bioactive compounds and nutraceutical properties of Goji berry (*Lycium barbarum* L.) .Appl. Sci. 15(1) (2024). <https://doi.org/10.3390/app15010262>
48. S. Abdullah, R.C. Pradhan, D. Pradhan, S. Mishra, Modeling and optimization of pectinase-assisted low-temperature extraction of cashew apple juice using artificial neural network coupled with genetic algorithm .Food Chem. 339 (2021). <https://doi.org/10.1016/j.foodchem.2020.127862>
49. A. Rodríguez-Silva, L.A. Ochoa-Martínez, S.M. González-Herrera, O.M. Rutiaga-Quiñones, R.F. González-Laredo, B. Olmedilla-Alonso, S. Vega-Maturino, Coloring potential of anthocyanins from purple sweet potato paste: Ultrasound-assisted extraction, enzymatic activity, color and its application in ice pops. Food Chem. Adv. 3 (2023). <https://doi.org/10.1016/j.focha.2023.100358>
50. M.F. Manzoor, N. Ahmad, Z. Ahmed, R. Siddique, A. Mehmood, M. Usman, X.A. Zeng, Effect of dielectric barrier discharge plasma, ultra-sonication, and thermal

processing on the rheological and functional properties of sugarcane juice. J. Food Sci. 85(11) (2020). <https://doi.org/10.1111/1750-3841.15498>

51. D. Saxena, L. Sabikhi, S.K. Chakraborty, D. Singh, Process optimization for enzyme aided clarification of watermelon juice J. Food Sci. Technol. 51 (2014). <https://doi.org/10.1007/s13197-012-0720-1>

52. G. Panda, K. Vivek, S. Mishra, R.C. Pradhan, Characterization and optimization of process parameters for enzyme assisted extraction of kendu (*Diospyros Melanoxylon Roxb.*) fruit juice. Int. J. Fruit Sci. 21(1) (2021). <https://doi.org/10.1080/15538362.2021.1873220>

53. L.F.R. da Silva, A.D.S. Gomes, D.R.G. Castro, F.D.C.D.A. Souza, J.M. Mar, L.S. da Silva, P.H. Campelo, Ultrasound-assisted homogenization and gum Arabic combined to physicochemical quality of cupuaçu juice J. Food Process. Preserv. 43(9) (2019). <https://doi.org/10.1111/jfpp.14072>

54. E. Radziejewska-Kubzdela, A. Szwengiel, H. Ratajkiewicz, K. Nowak, Effect of ultrasound, heating and enzymatic pre-treatment on bioactive compounds in juice from *Berberis amurensis* Rupr Ultrasonics. Sonochemistry. 63 (2020) 104971. <https://doi.org/10.1016/j.ultsonch.2020.104971>

55. A. Görgüç, C. Bircan, F.M. Yılmaz, Sesame bran as an unexploited by-product: Effect of enzyme and ultrasound-assisted extraction on the recovery of protein and antioxidant compounds. Food Chem. 283 (2019). <https://doi.org/10.1016/j.foodchem.2019.01.077>

56. J. Wang, S.K. Vanga, V. Raghavan, High-intensity ultrasound processing of kiwifruit juice: Effects on the ascorbic acid, total phenolics, flavonoids and antioxidant capacity. LWT. 107 (2019). <https://doi.org/10.1016/j.lwt.2019.03.024>

57. D.D.R.C. Dias, Z.M.P. Barros, C.B.O. de Carvalho, F.A. Honorato, N.B. Guerra, P.M. Azoubel, Effect of sonication on soursop juice quality. LWT-Food Sci. Technol. 62(1) (2015). <https://doi.org/10.1016/j.lwt.2014.09.043>
58. Y. Chen, Y. Hong, D. Yang, Z. He, X. Lin, G. Wang, W. Yu, Simultaneous determination of phenolic metabolites in Chinese citrus and grape cultivars. PeerJ. 8 (2020). <http://dx.doi.org/10.7717/peerj.9083>
59. A. Slatnar, J. Jakopic, F. Stampar, R. Veberic, P. Jamnik, The effect of bioactive compounds on in vitro and in vivo antioxidant activity of different berry juices. PLoS One. (2012). <https://doi.org/10.1371/journal.pone.0047880>
60. X. Zhou, W. Wang, M. Ma, E. Xu, D. Liu, Ultrasonication of thawed Huyou juice: Effects on cloud stability, physicochemical properties and bioactive compounds. Foods. 10(8) (2021). <https://doi.org/10.3390/foods10081695>

Table 1. Box- Behnken design used to optimize USEZT conditions and analyse the results for three selected response variables

Independent variables					Responses		
Run	US time	Amplitude	Enz con	Incubation	Phenol	Acn	DPPH
(min)	(min)	(%)	(%)	time (min)	(mg/100g)	(mg/100g)	(%)
	X ₁	X ₂	X ₃	X ₄			
1	30.00 (-1)	30.00 (-1)	0.03 (0)	45.00 (0)	678	36.00	61.18
2	20.00 (1)	30.00 (-1)	1.01(0)	45.00 (0)	774	38.84	69.99
3	20.00 (-1)	40.00 (1)	1.01(0)	60.00 (0)	737	37.72	64.39

4	20.00 (1)	40.00 (1)	0.03 (0)	45.00 (0)	733	37.67	64.33
5	30.00 (0)	30.00 (0)	1.01(-1)	30.00 (-1)	689	36.09	61.66
6	20.00 (0)	40.00 (0)	1.01 (1)	30.00 (-1)	739	37.78	64.56
7	20.00 (0)	30.00 (0)	1.01(-1)	45.00 (1)	766	37.78	69.05
8	10.00 (0)	30.00 (0)	1.01(1)	30.00 (1)	705	36.91	62.35
9	20.00 (-1)	30.00 (0)	1.01 (0)	45.00 (-1)	759	38.79	69.22
10	30.00 (1)	40.00 (0)	1.01(0)	45.00 (-1)	661	35.13	60.31
11	20.00 (-1)	30.00 (0)	1.01(0)	45.00 (1)	764	38.69	69.75
12	20.00 (1)	30.00 (0)	2.00 (0)	60.00(1)	728	37.52	64.05
13	10.00 (0)	30.00 (-1)	0.03(-1)	45.00 (0)	700	36.65	62.18
14	20.00 (0)	30.00 (1)	0.03 (-1)	60.00 (0)	758	38.64	68.53
15	30.00 (0)	30.00 (-1)	1.01(1)	60.00 (0)	682	36.04	61.36
16	10.00 (0)	40.00 (1)	1.01 (1)	45.00 (0)	670	35.74	61.18
17	30.00 (-1)	30.00 (0)	2.00 (-1)	45.00 (0)	675	35.48	60.13
18	20.00 (1)	20.00 (0)	2.00 (-1)	45.00 (0)	743	37.93	64.90
19	20.00 (-1)	20.00 (0)	1.01(1)	30.00 (0)	750	38.28	67.83
20	20.00 (1)	20.00 (0)	1.01(1)	60.00 (0)	747	38.10	67.48
21	20.00 (0)	30.00 (-1)	2.00 (0)	30.00 (-1)	731	37.62	64.22
22	20.00 (0)	30.00 (1)	0.03 (0)	30.00 (-1)	761	38.69	68.83
23	10.00 (0)	30.00 (-1)	1.01 (0)	60.00 (1)	698	36.71	62.00
24	10.00 (0)	30.00 (1)	2.00 (0)	45.00 (1)	666	35.59	60.47
25	30.00 (0)	20.00 (0)	1.01 (0)	45.00 (0)	694	36.15	61.88
26	20.00 (0)	20.00 (0)	0.03 (0)	45.00 (0)	746	37.98	67.13
27	20.00 (0)	30.00 (0)	1.01(0)	45.00 (0)	766	38.74	69.05
28	20.00 (0)	40.00 (0)	2.00 (0)	45.00 (0)	745	37.47	64.02

29	10.00(0)	20.00(0)	1.01(0)	45.00(0)	708	37.21	62.86
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Table 2. Analysis of variance (ANOVA) for the regression model of RSM for USEZT jamun pulp process

Response	TPC			Acn			DPPH		
Source	Sum of squares	F Value	P value	Sum of squares	F Value	P Value	Sum of squares	F Value	P value
Model	32602.9	14.1025	< 0.0001	33.3928	12.2865	< 0.0001	257.161	5.73693	0.0012
A-US Time	27904.3	168.982	< 0.0001	27.2764	140.505	< 0.0001	206.353	64.4485	< 0.0001
B-Amplitude	131.352	0.79544	0.3875	0.18208	0.93791	0.3493	0.38056	0.11886	0.7354
C-Enz Con	16.6004	0.10053	0.7559	0.14547	0.74934	0.4013	2.156	0.67337	0.4256
D-Incb Time	275.959	1.67114	0.217	0.39747	2.0474	0.1744	2.12377	0.6633	0.429
AB	52.742	0.31939	0.5809	0.09195	0.47366	0.5026	0.17389	0.05431	0.8191
AC	97.6037	0.59107	0.4548	0.00734	0.03781	0.8486	0.00654	0.00204	0.9646
AD	285.287	1.72763	0.2098	0.4017	2.06923	0.1723	2.14663	0.67044	0.4266
BC	18.4285	0.1116	0.7433	0.21658	1.11563	0.3087	0.14034	0.04383	0.8372
BD	752.432	4.55655	0.051	0.87111	4.48724	0.0525	5.51043	1.72103	0.2107
CD	0.0013	7.90E-06	0.9978	0.00069	0.00355	0.9533	0.00382	0.00119	0.9729

935	A ²	25576.8	154.887	< 0.0001	23.4885	120.993	< 0.0001	192.368	60.0807	< 0.0001
936	B ²	836.479	5.06552	0.041	1.73984	8.96216	0.0097	4.16014	1.29931	0.2735
	C ²	352.623	2.13541	0.166	0.05822	0.2999	0.5926	8.15522	2.54706	0.1328
	D ²	46.8831	0.28391	0.6025	0.26425	1.3612	0.2628	0.41768	0.13045	0.7234
	Lack of Fit	2110.68	5.82899	0.0333	2.14494	2.08001	0.2173	39.7715	4.37184	0.0594
	CV %	1.78			1.18			2.77		
	Adqprecision	10.35			10.35			7.44		
	R square	0.93			0.92			0.85		
	Adj R square	0.86			0.84			0.70		

Table 3. Physiochemical analysis of control and optimized pulp samples

Parameters	Control pulp	Synergistic effect of USEZT pulp
pH	3.46± 0.05	3.20± 0.02
TSS (°B)	8.10 ± 1.15	9.00 ± 1.00
Moisture (%)	92.12 ± 0.62	95.43± 0.12
Ash (%)	2.07 ± 0.02	2.05± 0.01
Viscosity (mPa.S)	1.87 ± 0.01	1.66± 0.01
Juice Yield (%)	73.00 ± 0.05	75.5 ± 0.07
Acidity (%)	0.54 ± 0.01	1.38± 0.05
Ascorbic acid (mg/100g)	22.69 ± 0.42	24.91± 0.42
Protein (g/100mL)	0.37 ± 0.05	0.34± 0.10
Dietary fibre (g/100mL)	1.10 ± 0.11	0.87± 0.17
Total sugar (%)	8.19 ± 0.10	8.24± 0.11
Reducing sugar (%)	6.60± 0.02	6.67± 0.11
ABTS Assay (%)	45.58 ± 0.05	45.58 ± 0.09
Reducing power (µg/mL)	70.42 ± 0.73	70.63± 0.62
Gallic acid (mg/100g)	37.39± 0.52	42.10 ± 0.55
Ferulic acid (mg/100g)	0.73± 0.01	0.91 ± 0. 08
Quercetin (mg/100g)	0.48 ± 0.02	0.80 ± 0.05
L*	8.54± 0.17	6.17 ± 0.02
a*	26.22 ± 0.01	31.29± 0.02
b*	-6.91 ± 0.02	-7.66± 0.15
Colour density	1.88 ± 0.10	2.30 ± 0.09

* **Note:** The results were expressed as mean ± standard deviations

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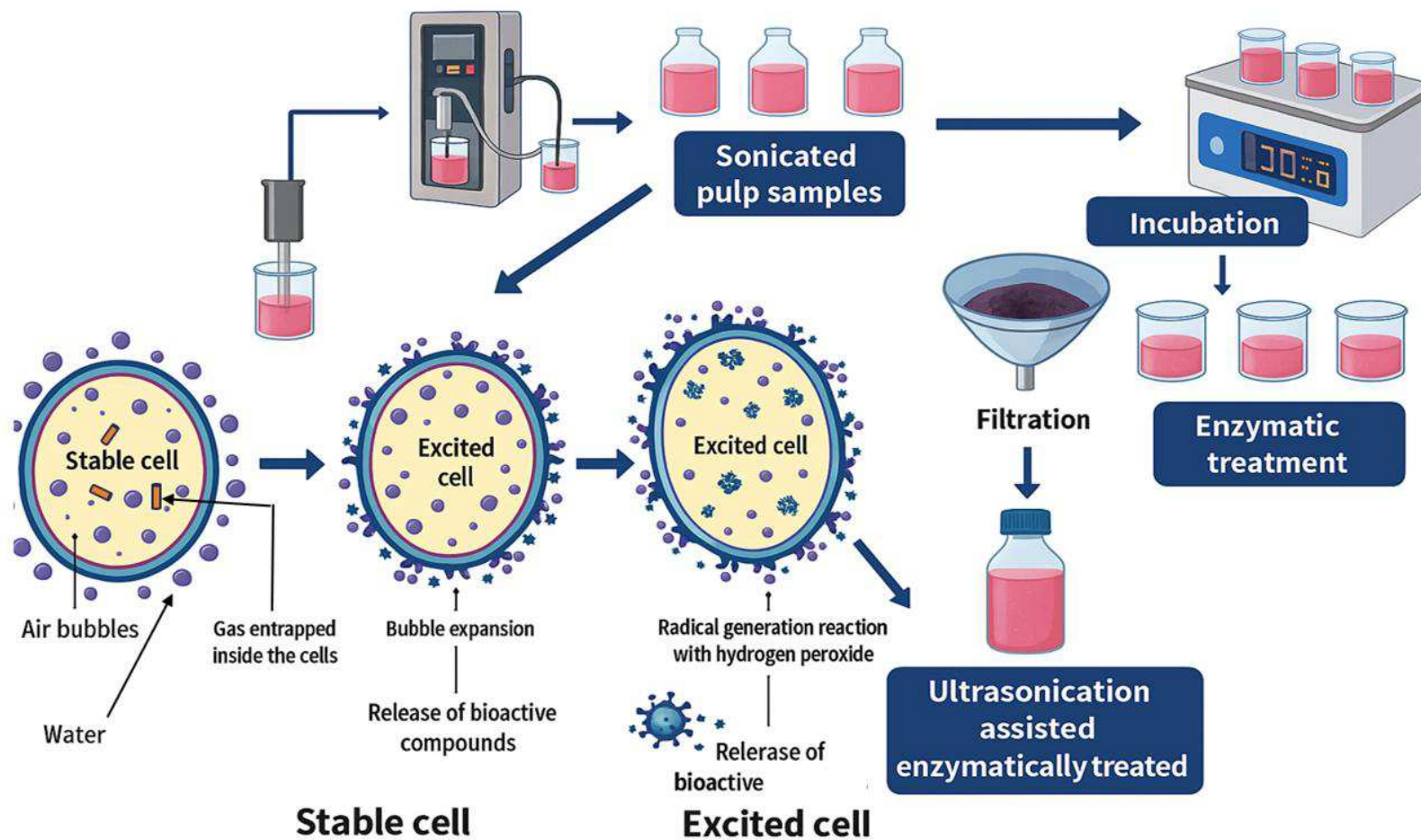


Fig. 1. Proposed mechanism describing sonication action on bioactive compounds present in jamun pulp

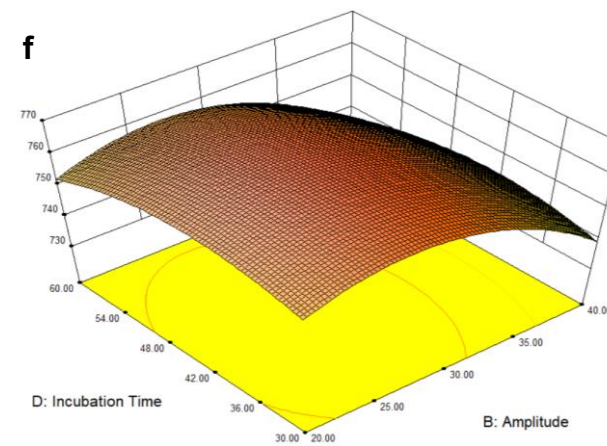
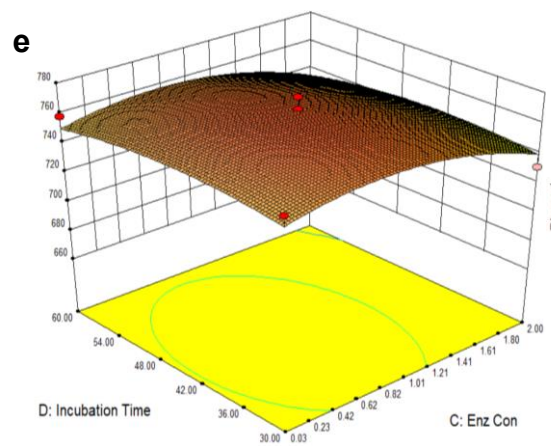
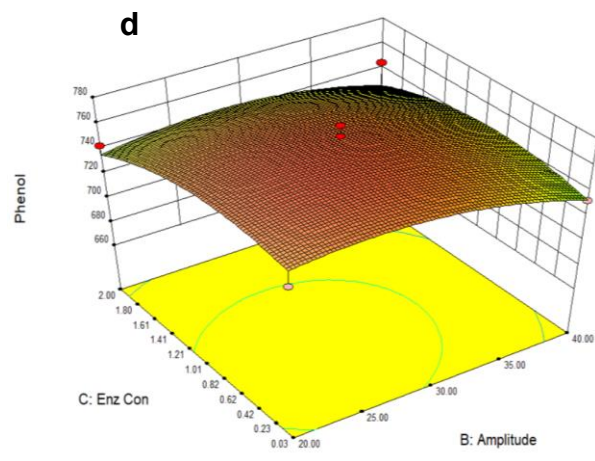
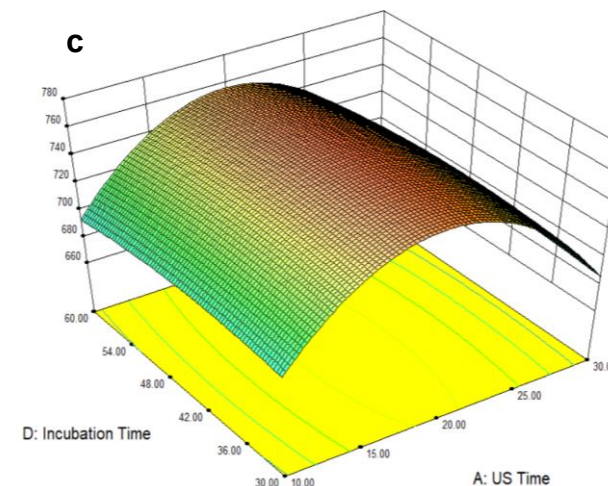
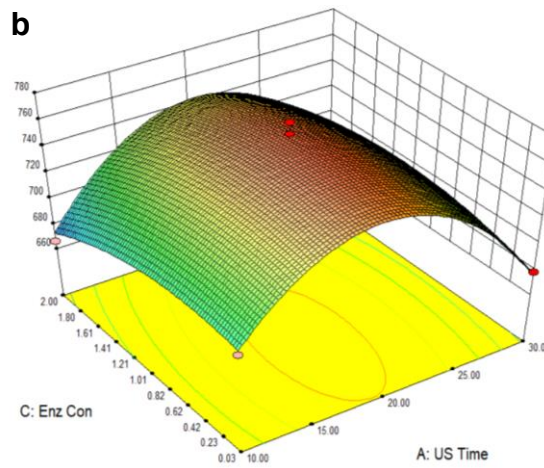
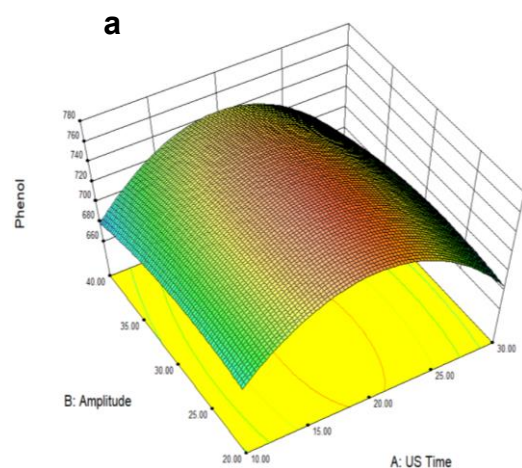
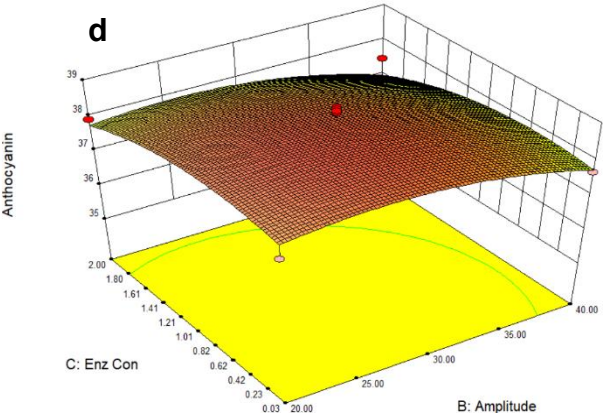
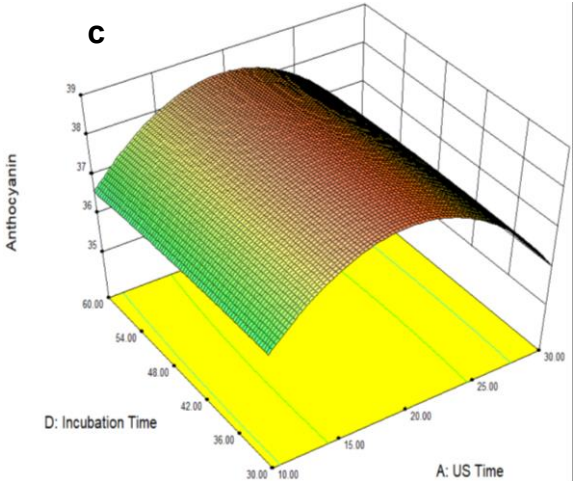
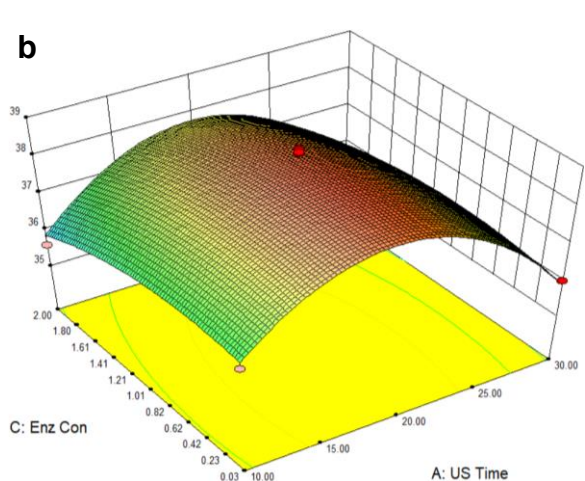
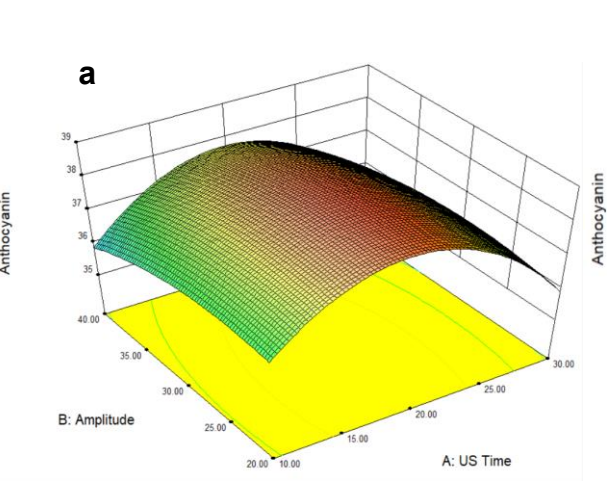
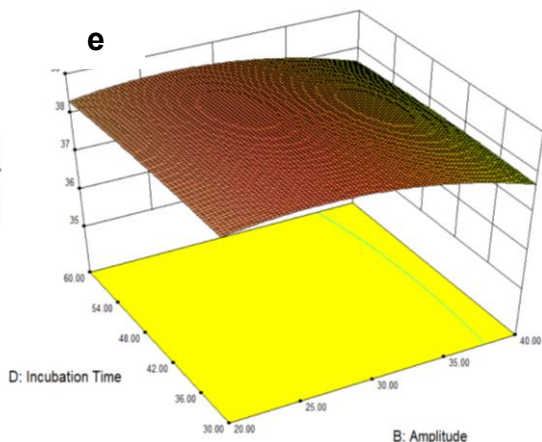
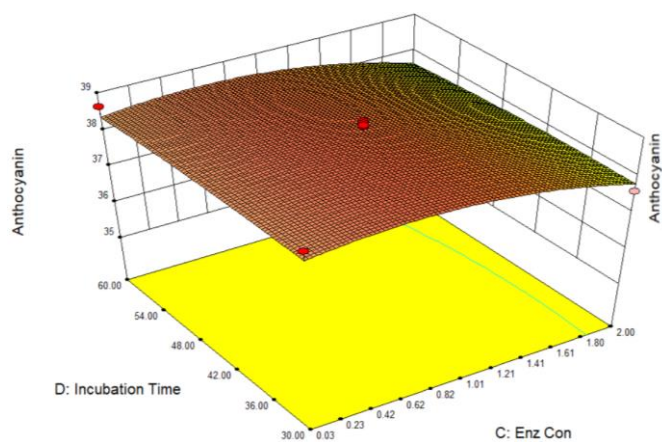


Fig. 2. Response surface 3D plots demonstrating the interaction effect of independent variables for the extraction of phenolic compounds from USEZT treated jamun pulp

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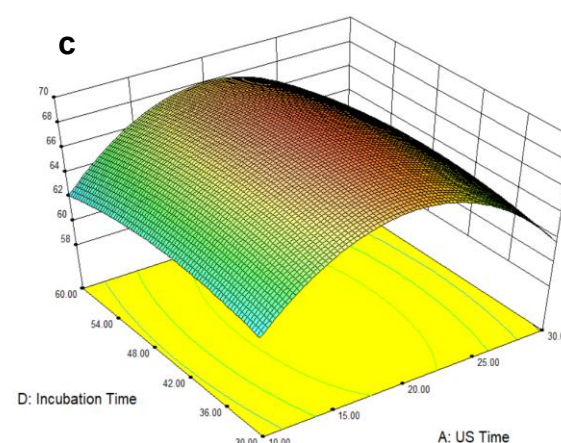
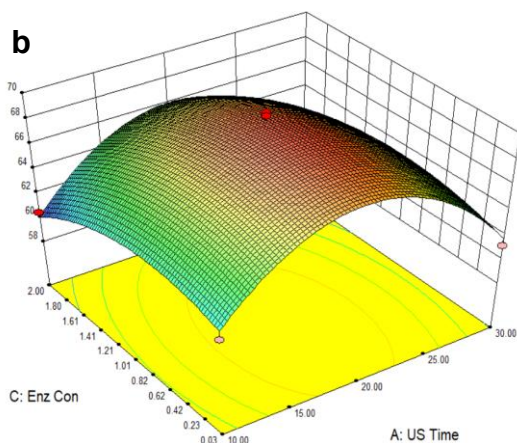
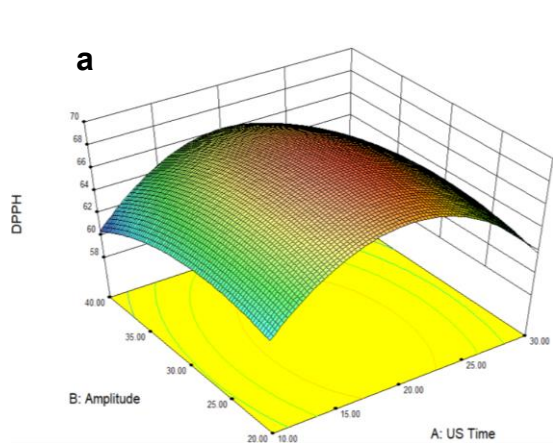
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952 **Fig. 3.** Response surface 3D plots demonstrating the interaction effect of independent variables for the extraction of Acn from USEZT treated
953 jamun pulp



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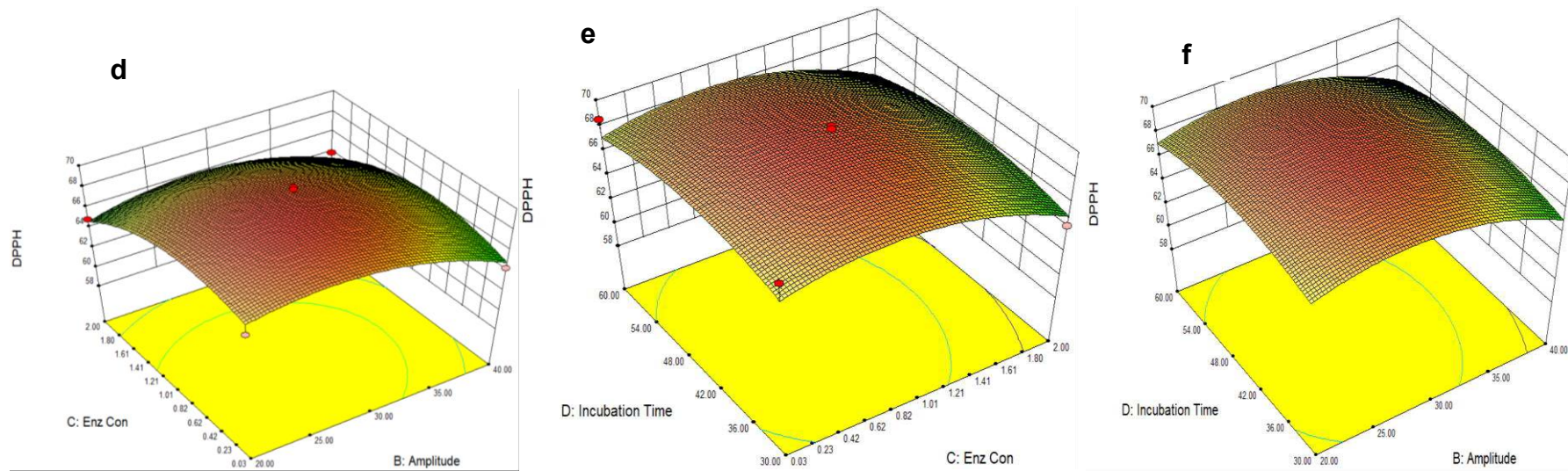


Fig. 4. Response surface 3D plots demonstrating the interaction effect of independent variables for the estimation of DPPH scavenging activity from USEZT treated jamun pulp

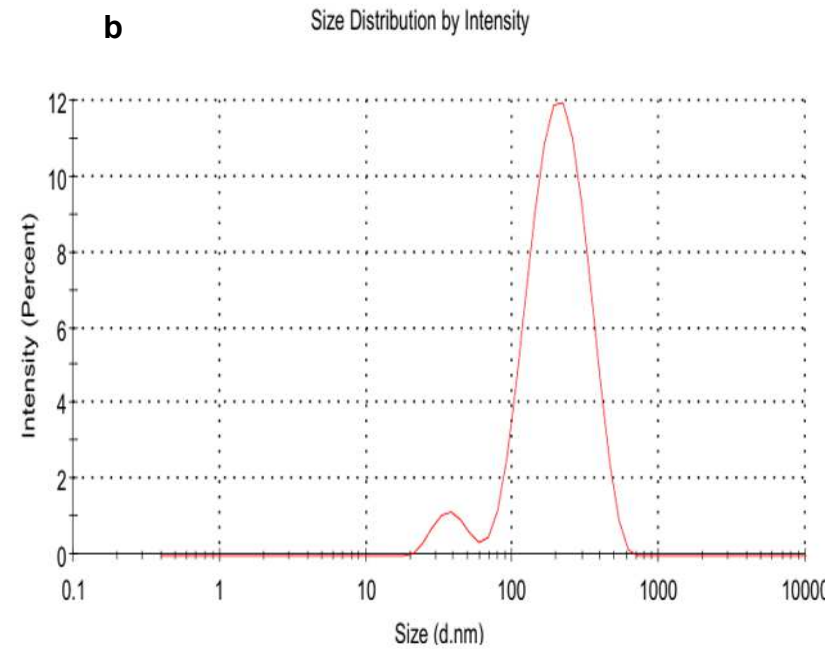
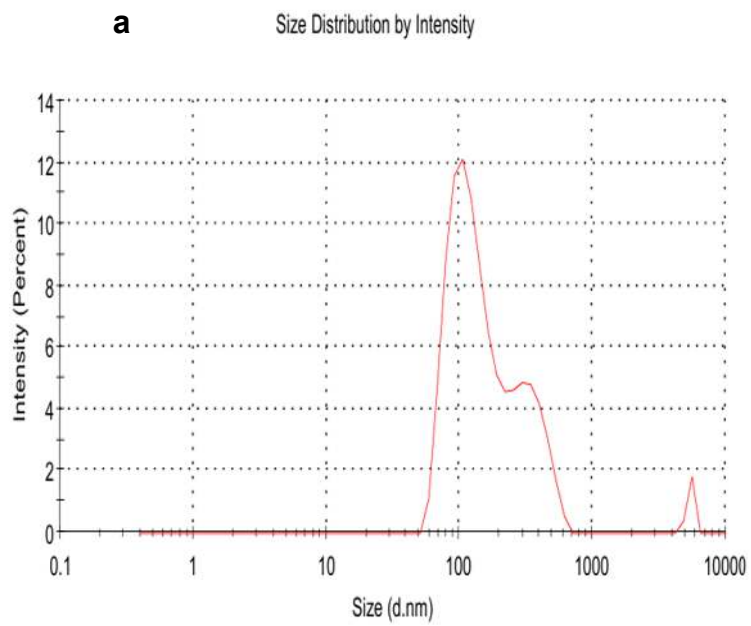
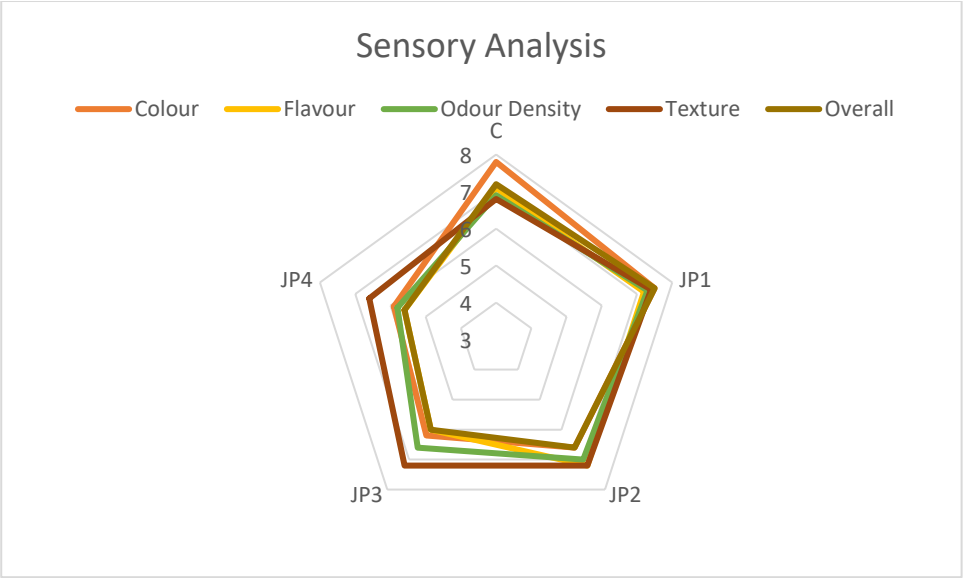


Fig. 5. Average particle size (a) Control and (b) Optimized jamun pulp samples

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968 **Fig. 6.** Spider chart for sensory evaluation of the jamun pulp samples

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