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Effect of high-pressure soaking on the physicochemical, nutritional, and techno-functional properties of pearl millets

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Pearl millets are important crops that have a significant nutritional value. Using processed pearl millets can add value to various food products and contribute to food and nutritional security. In this study, high pressure processing was applied in soaking the pearl millets to increase iron bioavailability by degrading the anti-nutrient phytate. Millet samples were soaked at three pressure levels of 300, 350, and 400 MPa for three different treatment times of 30-, 60-, and 90 min. Treatments resulted in up to an 80.61% reduction in the phytate content. The free iron content of the pearl millet increased to 579.24 mg kg^{-1} from 127.73 mg kg^{-1} with a pressure of 350 MPa for 90 min soaking. There were no significant changes in the nutritional profile of the treated samples, except that longer treatment times reduced the carbohydrate content. High-pressure treatments resulted in up to 12.1% and 5.9% increase in water and oil absorption capacities of millets, respectively. Significant differences in thermal and pasting properties of treated samples of millets showed that high-pressure processing altered the techno-functional properties of millets. Results illustrate that the high-pressure treatment, as a novel approach, can reduce phytate and improve free iron content in millets.

Sustainability spotlight

High-pressure processing of pearl millet enhances its nutritional profile by reducing anti-nutrient phytate significantly and increasing iron bioavailability, supporting food and nutritional security without compromising its nutritional integrity. This innovative approach promotes sustainable agricultural practices by improving the utilization of a climate-resilient crop, contributing to resilient food systems and improved public health outcomes.

1. Introduction

Millets are a rich source of calcium, zinc, iron, lipids, energy,[1] and they also have high-quality proteins that are gluten-free and have a lower glycemic index (GI) due to their slower-digesting starch.[2] The utilization of millets has attracted extensive attention in the food sector due to consumers' desire for health-promoting food products. The top producers of millets reported for the year 2023–2024 are India, with 12.2 million metric tons (MT), followed by Niger with a production of 3.16 million MT, and China has 2.7 million MT production.[3] The Poaceae family's pearl millet (*Pennisetum glaucum*), also known as Bajra, Bajri, Kambu, Kamban, Sajje, Sajjalu, and other regional names in local languages, is a multipurpose cereal crop. It is traditionally used as a food, feed, forage, and stover crop.[3] Pearl

millets are ranked as the sixth most important cereal crop with a global millet cultivation of more than 30 million ha.[4] Pearl millet is the main diet for over 90 million people in the Sahelian regions of Africa and the northwestern parts of India.[5] In a few of the world's hottest and driest farmed areas, pearl millet is crucial for food security. Pearl millet is extremely nutritious compared to other grains, such as wheat, rice, and maize, with high fat, protein, and mineral contents being a rich source of iron, zinc, magnesium, copper, manganese, potassium, and phosphorus.[6] It comprises 92.5% dry matter, 2.1% ash, 2.8% crude fiber, 7.8% crude fat, 13.6% crude protein, and 63.2% starch.[7]

Due to its limited availability as a convenient food with an exceptional nutritional profile, pearl millet has been accessible to traditional consumers. One of the primary causes of its underutilization is the presence of anti-nutrients, which reduce carbohydrate and protein digestibility and diminish mineral bioavailability. Phytin, tannins, and polyphenols are some of the anti-nutritional factors in pearl millet.[8] These combine with compounds or synthetic substances causing impairment in nutrient absorption, digestion, and consumption.[9] Phytic acid (in the form of phytate phosphorus) is the main anti-nutrient in

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pearl millets, depending on the cultivated variety, which varies from 179 to 306 mg/100 g.[10] It forms compounds with dietary minerals like iron, zinc, and calcium in the human stomach, limiting dietary mineral absorption. Phytic acid inhibits the action of proteolytic and amylolytic enzymes, preventing protein degradation.[11] Phytic acid (IP6) is a highly reactive acidic compound that readily binds mineral cations, and in this complexed form is called phytin.[12] Previous studies proved that the iron content of the whole grain, bran, and endosperm was 5.0–6.4, 3.1–4.7, and 4.1–5.8 mg/100 g, but the bioaccessibility of iron in pearl millets was only 0.16–0.44, 0.16–0.20, and 0.20–0.48 mg/100 g, respectively.[13] One-third of the global total population is stricken by iron deficiency, which is caused by the lack of iron intake compared to the recommended dietary allowance (RDA). The primary reason is they rely on staple cereal foods and vegetarian eating patterns with poor iron bioavailability. The RDA for iron is 10 mg for children, 8 mg for males, and 18 mg for women.[6] In order to maximize the absorption of extractable iron by the body, it is necessary to reduce the phytate in the foods.[14]

Various processing methods and innovative approaches have been investigated to enhance mineral availability by phytic acid reduction. Soaking is a common pre-treatment for cooking, germination, fermentation, and processing of cereal grains. The reduction of anti-nutritional compounds is affected by soaking temperature, time, and medium used. During soaking, the phytase enzyme is activated and aids in phytate reduction. About 2–23% increase in *in vitro* solubility and availability of iron (Fe) was observed by soaking grains.[15]

High-pressure processing causes stress on the cells, partially degrading the cell's structure. This activates a few enzymes that reduce the anti-nutritional factors.[16] Treatment of buckwheat at 600 MPa for 30 min resulted in a reduction of 45.5% phytin, 13% trypsin inhibitor, 19.9% tannin, and 4.5% saponin contents.[17] Foxtail millets were treated at a pressure of 600 MPa and 60 °C for 120 min and the tannin and phytin content reached negligible levels of 0.1870% and 0.0006%, respectively, analyzed using the vanillin–HCl method and phytic assay kit. Treatment of 25 g of germinated foxtail millets at 400 MPa at 40 °C for 30, 60, 90, and 120 min showed a maximum reduction in phytic acid of about 67.87%. Furthermore, the phytic acid reduction was the least when soaking time and temperature increased at an atmospheric pressure.[18]

Pearl millets are underutilized and economic starch sources, constituting up to 70% starch in the grain. Previous studies showed that pearl millet starch has better techno-functional qualities than other cereals.[19] Techno-functional characteristics including gelatinization, pasting properties, solubility, and absorption capacity may be altered by physical or mechanical modifications for a wider range of food applications.[19] High pressure processing allows rapid water diffusion until starch gelatinisation and high pressure soaking significantly improves the nutritive value of grains. This helps in processing grains in less time and allows the exploration of grain utilization in various food products as ingredients. The aim of this study was to investigate the impact of high-pressure soaking of pearl millets on the phytate reduction and enhancement of the free

bioavailability of iron. The techno-functional characteristics of pearl millet with high-pressure soaking were also examined to broaden its use in ready-to-cook and ready-to-eat items.

2. Materials and methods

2.1. Materials

Pearl millets (*Pennisetum glaucum*) were purchased from a local supermarket in Thanjavur, Tamil Nadu, India. Pearl millets were sealed in polyethylene pouches and stored at 25 °C in a dry space until use. All the chemicals utilized in the analysis were purchased from HiMedia Laboratories and were of analytical grade.

2.2. High-pressure processing equipment

The high-pressure processing machine (model: HPP 600 MPa) with a dimension of 2000 mm × 1350 mm × 1960 mm and a capacity of 5 L was supplied by KK Lifesciences, Chennai, India. The maximum working pressure was 600 MPa, with water used as a medium of pressure transmission in the vessel. The pressure and temperature conditions inside the vessel were monitored continuously during the process in the control panel. Typically, it takes up to 120 seconds to reach the target pressure, while pressure release occurs within 10 seconds. As the pressure increases, the temperature of the transmitting fluid rises by approximately 3 ± 1 °C for every 100 MPa increment. At the final pressure level, the temperature is expected to stabilize at 22 ± 5 °C and remain constant throughout the treatment. The reported treatment durations for the various combinations in this study exclude the time required for pressurization and depressurization.

2.3. Treatment of pearl millets

A polythene pouch containing 25 g pearl millet and 150 mL distilled water was vacuum-sealed. The pouches were soaked at three high-pressure levels (300, 350, and 400 MPa) each for three different periods (30, 60, and 90 min). The water was entirely drained from the polyethylene pouches after treatment, and the millets were subsequently dried at 50 °C for 3 h. The pearl millet sample was soaked using the same procedure at room temperature (30 ± 2 °C) for 90 min, the highest treatment time used among treatments without high pressure was used as the control. The control and dried treated millets were ground and sieved using 40 mesh size (particle size ≤ 420 μm) sieves for further analysis.

2.4. Determination of the colour of pearl millets

Colour values (L^*, a^*, and b^*) of the control and treated grains of pearl millets were assessed in a Hunter lab colour flex meter (Hunter Associates Laboratory, Inc., Reston, Virginia, USA). It was calibrated using a standardized black and white tile. The millets were placed in the cup provided in the calorimeter, and the L^*, a^*, and b^* values were recorded for multiple mixtures of millets. In the L^*, a^*, and b^* colour system, the L^* (lightness) value 0 to 100 represents black to lightness, the a^* value from $-a$ to $+a$ indicates green to red, and the b^* value from $-b$ to $+b$

Table 1 Colour values of pearl millets treated with high-pressure processing[a]

Pressure (MPa)	Treatment time (min)	$L*$	$a*$	$b*$	ΔE
0 (control)	—	53.80 ± 0.26^a	2.63 ± 0.13^e	21.72 ± 0.15^b	—
0 (soaked)	90	50.77 ± 0.85^b	3.89 ± 0.12^{abc}	21.45 ± 0.74^b	3.35 ± 0.80^c
300	30	50.19 ± 0.59^{bc}	3.16 ± 0.20^{de}	20.58 ± 0.24^b	3.83 ± 0.59^{bc}
	60	50.74 ± 0.45^b	3.26 ± 0.14^{cde}	20.60 ± 0.48^{bc}	3.34 ± 0.47^c
	90	48.32 ± 1.00^d	3.28 ± 0.31^{cd}	20.47 ± 0.40^{bc}	5.65 ± 0.99^a
350	30	49.80 ± 0.15^{bcd}	3.38 ± 0.29^{bcd}	20.79 ± 0.67^{bc}	4.21 ± 0.27^{abc}
	60	49.15 ± 0.701^{bcd}	3.54 ± 0.30^{bcd}	20.71 ± 0.41^{bc}	4.86 ± 0.69^{abc}
	90	48.78 ± 0.45^{cd}	3.74 ± 0.36^{abcd}	21.31 ± 0.759^b	5.20 ± 0.48^{ab}
400	30	50.02 ± 0.95^{bc}	3.44 ± 0.11^{bcd}	19.72 ± 0.61^c	4.44 ± 0.56^{abc}
	60	50.11 ± 0.16^{bcd}	4.30 ± 0.14^a	23.32 ± 0.51^a	4.37 ± 0.28^{abc}
	90	48.47 ± 0.41^{cd}	3.93 ± 0.11^{ab}	21.18 ± 0.18^b	5.51 ± 0.41^{ab}

[a] Values are represented as mean $\pm$ SD. Values in the same column with different lowercase letters differed significantly as determined by ANOVA followed by Tukey's test ($p < 0.01$).

represents blue to yellow. The measurements were done in triplicate. The ΔE (colour difference) between the treated and the control sample was evaluated using the following formula:

$$\Delta E = \sqrt{(L* - L)^2 + (a* - a)^2 + (b* - b)^2};$$

where $L*$, $a*$, and $b*$ represent the colour values of the control samples and L, a, and b represent the colour values of the treated samples. The values are reported in Table 1.

2.5. Texture analysis of pearl millets

Texture analysis of the millets was executed using the Texture Analyzer TA.XT plus (Stable Micro System Ltd, Surrey, UK). A P/35 (35 mm diameter) cylinder aluminium probe was used to measure the firmness of millets by using compression test mode. A trigger force of 5.0 g with a strain of 50% was applied in the measurement with a test speed of 0.50 mm s^{-1} at a distance of 5.00 mm, 0.50 mm s^{-1} pre-test speed, and 10.00 mm s^{-1} post-test speed. The test was repeated for ten different pearl millet kernels of each treatment. The firmness values are reported in Table 2.

2.6. Determination of nutritional properties

The protein and fat content were determined[20] with the Kjeldahl and Soxhlet apparatus, respectively. Ash content was determined by incinerating the samples in a muffle furnace at a temperature of 550 °C for 5.5 h.[20] The moisture content was measured using a moisture meter (Mettler Toledo HE53, Greifensee, Switzerland). The carbohydrate was calculated using the equation

Carbohydrate% = $100\% - (fat\% + protein\% + ash\% + moisture\%)$

The nutritional content values are reported in Table 3.

2.7. Determination of phytate content

The phytate content of the control and treated pearl millet samples was determined colorimetrically.[21] A 2 g millet powder was extracted with 30 mL of 3% trichloroacetic acid for 30 min by mechanical shaking. After centrifugation at 2000 rpm for 15 min at 4 °C, 4 mL of ferric chloride solution (583 mg of FeCl$_3$ dissolved in 100 mL of trichloroacetic acid) was added into a 10 mL aliquot

Table 2 Effect of high-pressure processing on the techno-functional properties of pearl millets[a]

Pressure (MPa)	Treatment time (min)	Firmness (g)	Water solubility (g g^{-1})	WAC (%)	OAC (%)
0 (control)	—	4338 ± 565^{bc}	0.05 ± 0.02	226.10 ± 4.86^{bcde}	204.51 ± 2.86
0 (soaked)	90	6084 ± 2576^{bc}	0.06 ± 0.04	229.51 ± 0.17^{bc}	212.69 ± 0.80
300	30	3742 ± 1044^c	0.06 ± 0.00	211.59 ± 3.61^f	203.19 ± 4.17
	60	4406 ± 2399^c	0.05 ± 0.01	234.22 ± 5.67^b	207.05 ± 2.30
	90	4988 ± 1419^{bc}	0.03 ± 0.01	253.45 ± 8.15^a	204.66 ± 5.51
350	30	5239 ± 1438^{bc}	0.05 ± 0.02	226.75 ± 1.19^{bcd}	198.93 ± 2.19
	60	5608 ± 1875^{bc}	0.04 ± 0.01	218.27 ± 0.72^{def}	209.84 ± 3.25
	90	$21\,355 \pm 5469^a$	0.02 ± 0.00	226.75 ± 0.98^{bcd}	225.80 ± 35.00
400	30	5345 ± 1950^{bc}	0.05 ± 0.01	222.51 ± 1.41^{cde}	197.21 ± 1.42
	60	6737 ± 1362^{bc}	0.05 ± 0.01	226.48 ± 0.91^{bcde}	211.86 ± 4.33
	90	$10\,107 \pm 6859^b$	0.03 ± 0.01	216.12 ± 1.23^{ef}	216.74 ± 1.99

[a] Values are represented as mean $\pm$ SD. Values in the same column with different lowercase letters differed significantly as determined by ANOVA followed by Tukey's test ($p < 0.01$). WAC: water absorption capacity; OAC: oil absorption capacity.

Table 3 Physicochemical properties of pearl millets soaked at high-pressure[a]

Pressure (MPa)	Treatment time (min)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)
0 (control)	—	9.87 ± 0.53^f	10.51 ± 1.16^{ab}	4.46 ± 0.76	1.43 ± 0.03^a	73.73 ± 1.91^{ab}
0 (soaked)	90	29.44 ± 0.67^{abc}	12.55 ± 1.26^a	5.22 ± 0.62	1.27 ± 0.34^{ab}	51.53 ± 1.37^b
300	30	31.05 ± 0.84^{ab}	10.21 ± 0.25^{ab}	5.52 ± 0.43	0.99 ± 0.00^b	52.22 ± 1.46^{ab}
	60	26.87 ± 0.21^{cde}	8.90 ± 0.51^b	5.25 ± 0.17	0.92 ± 0.00^b	58.06 ± 0.40^a
	90	26.46 ± 0.36^{cde}	12.84 ± 0.25^a	5.16 ± 0.59	0.96 ± 0.01^b	54.58 ± 0.09^b
350	30	32.58 ± 0.61^a	10.79 ± 2.24^{ab}	4.94 ± 1.50	0.98 ± 0.01^b	50.71 ± 0.35^{ab}
	60	23.76 ± 0.77^e	10.21 ± 1.77^{ab}	5.11 ± 0.75	0.99 ± 0.00^b	59.93 ± 3.14^{ab}
	90	28.12 ± 0.34^{bcd}	11.53 ± 0.51^{ab}	5.34 ± 0.11	0.95 ± 0.01^b	54.05 ± 0.79^{ab}
400	30	25.27 ± 1.39^{de}	12.11 ± 1.34^{ab}	4.67 ± 0.33	1.41 ± 0.12^a	56.32 ± 2.60^b
	60	29.92 ± 3.31^{abc}	11.53 ± 0.25^{ab}	4.49 ± 0.10	0.98 ± 0.01^b	53.08 ± 3.65^{ab}
	90	29.84 ± 0.47^{abc}	10.92 ± 1.14^{ab}	4.50 ± 0.43	1.31 ± 0.30^{ab}	53.42 ± 0.51^{ab}

[a] Values are represented as mean $\pm$ SD. Values in the same column with different lowercase letters differed significantly as determined by ANOVA followed by Tukey's test ($p < 0.01$).

of supernatant. The mixture was heated in a boiling water bath for 45 min and then centrifuged again for 15 min. The supernatant was discarded, and the residue was washed multiple times by dispersing thoroughly in 25 mL of 3% trichloroacetic acid, continued heating for 5–10 min, and centrifuged. Again, washing in water was done. The pellet was dispersed in 3 mL of NaOH (1.5 N) and adjusted to 30 mL with deionized water, and boiled for 30 min. Whatman no. 2 retentive paper was used to filter the contents. The residue was rinsed with hot water, dissolved in 40 mL of hot HNO_3 (3.2 N), and adjusted to 100 mL with distilled water. From that, 5 mL was made up to 70 mL, and 20 mL of KSCN (1.5 M) was added. The absorption was read at 480 nm with a spectrophotometer. The colorimetric value is used to quantify the phytate phosphorus content, which is derived, presuming a fixed molecular ratio of 4 Fe : 6 P in the residue. The standard curve was obtained using ferric nitrate ($Fe(NO_3)_3$).[21] The phytate values and reduction percentage due to high-pressure processing are reported in Table 4.

2.8. Determination of free iron content

The free iron content was measured by evaluating the acid-digestible iron, which was extracted according to the

procedure of a previous study[22] with a minor modification. About 1 g of millet flour was extracted in 10 mL of 0.03 N HCl, equivalent to the acid content in the human stomach. A 0.5–1.0 g extracted sample was homogenized thoroughly and dried with 7 mL of concentrated HCl on a hot plate till a white/brownish red carbon-free ash was formed. The free iron in the residue was quantified using inductively coupled plasma-optical emission spectrometry.[23] The iron contents of different samples are represented as a bar graph chart (Fig. 1).

2.9. Techno-functional properties of high-pressure processed pearl millet

To understand the effect of soaking with high-pressure on the techno-functional properties, the water absorption, oil absorption, solubility, pasting, and thermal properties of the untreated and treated pearl millet flour were evaluated.

2.9.1. Water and oil absorption capacity. One gram of the pearl millet flour was suspended in 10 mL of deionized water for water absorption capacity (WAC) and oil for oil absorption capacity (OAC) in pre-weighed centrifuge tubes and mixed well for 30 s. The tubes were kept undisturbed for about 30 min under ambient conditions without stirring and then

Table 4 Effect of high-pressure treatment on phytate content in pearl millets[a]

Pressure (MPa)	Treatment time (min)	Phytate content (mg/100 g)	Reduction (%)
0 (control)	—	172.13 ± 7.50^a	—
0 (soaked)	90	$142.13 \pm.92^b$	17.43
300	30	104.63 ± 6.50^c	39.21
	60	102.13 ± 7.81^c	40.67
	90	84.63 ± 2.17^{cd}	50.83
350	30	98.38 ± 4.33^d	42.85
	60	75.38 ± 4.33^d	56.21
	90	73.38 ± 7.81^d	57.37
400	30	85.88 ± 15.00^{cd}	50.11
	60	37.13 ± 6.50^e	78.43
	90	33.38 ± 3.75^e	80.61

[a] Values are represented as mean $\pm$ SD. Values in the same column with different lowercase letters differed significantly as determined by ANOVA followed by Tukey's test ($p < 0.01$).
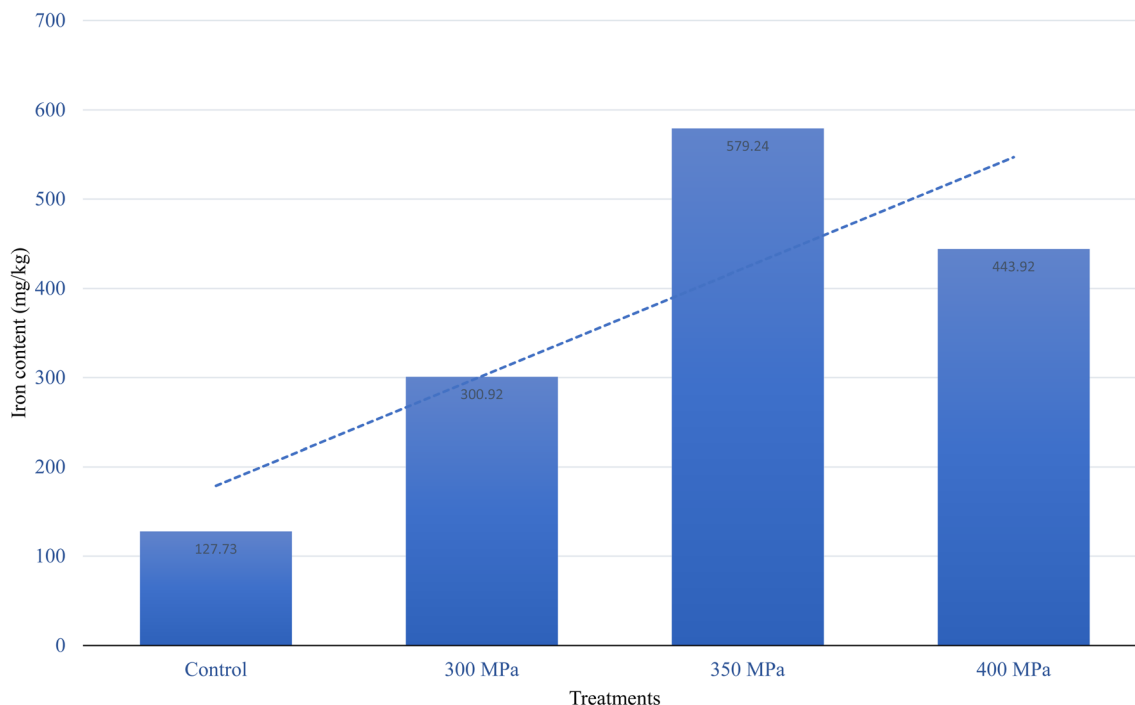
Fig. 1 Effect of high-pressure soaking for 90 min on the iron content of pearl millets.

centrifuged for 30 min at $1107 \times g$ force. The excess water or oil was discarded after centrifugation, and the precipitate was weighed. The WAC and OAC were calculated using the following equation.[24]

$$\text{WAC\% or OAC\%} = \frac{\text{weight of tubes with precipitate (g)} - \text{initial weight of tubes with sample (g)}}{\text{weight of the sample (g)}} \times 100$$

The values are reported in Table 2.

2.9.2. Water solubility. The water solubility of pearl millet flour was evaluated by adding 10 mL of deionized water into 0.5 g flour. The mixture was heated at 60 °C for 30 minutes without mixing. The obtained slurry was cooled to 25 °C and centrifuged for 10 min at $315 \times g$. The supernatant was collected with a pre-weighed dish, dried to evaporate the supernatant, and weighed.[25]

$$\text{solubility} = \frac{\text{weight of the soluble sample (g)}}{\text{weight of the sample taken (g)}}$$

The values are reported in Table 2.

2.9.3. Pasting properties. The pasting properties of the pearl millet flour were determined using a rheometer (Anton-Paar) with ST24-2D/2V/2V-30-SN64854 according to the procedures described by the AACC International method (2000). The flour sample (3 g, moisture content corrected to 14% w.b.) was dispersed in 25 mL deionized water. The flour suspensions were held at 50 °C for 1 min, linearly heated to 95 °C in 3 min 42 s

and kept at that temperature for 2 min 30 s, and then cooled to 50 °C in 3 min 48 s. Three replicates of the peak viscosity, final viscosity, pasting temperature, pasting time, hold strength, setback viscosity, and breakdown viscosity were recorded and are reported in Table 5.[26]

2.9.4. Differential scanning calorimetry (DSC). Thermal analysis of the control and 90 min treated samples at three levels of pressure (300, 350, and 400 MPa) was performed using DSC (DSC 3 STARe System, Mettler Toledo, Hong Kong) and is shown in Fig. 2.

4–6 mg (dry weight basis) of sample was weighed into an aluminium pan and deionized water was added to make suspensions with 75% moisture content. The pan was sealed hermetically and equilibrated for 15–18 h at room temperature. The instrument was calibrated using indium and an empty pan was used as a reference. The sample was scanned from 10 °C to 140 °C at a heating rate of 10 °C min^{-1}. Nitrogen gas was used as the purge gas at a 20 mL min^{-1} flow rate. The thermogram gives the values of onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) in °C. The gelatinization temperature range (T_R) was calculated as a difference between the gelatinisation peak's onset and conclusion temperature ($T_c - T_o$) and is reported in Table 6.

Table 5 Effect of high-pressure processing on the pasting properties of pearl millets[a]

Pressure (MPa)	Treatment time (mins)	Peak time (s)	Peak viscosity (cP)	Pasting temperature (°C)	Holding strength (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback from peak (cP)	Setback from trough (cP)
0 (control)	—	470.70 ± 8.24[e]	1027.40 ± 125.40[bc]	73.59 ± 0.75[a]	603.33 ± 14.45[d]	490.90 ± 37.40[ab]	1404.00 ± 80.70[c]	376.50 ± 55.30[a]	800.80 ± 66.90[de]
0 (soaked)	90	497.00 ± 1.21[ab]	963.40 ± 126.10[c]	74.96 ± 0.89[a]	673.80 ± 80.90[cd]	289.40 ± 44.90[b]	1367.30 ± 31.80[c]	404.50 ± 94.10[a]	693.90 ± 49.20[e]
300	30	499.80 ± 3.12[ab]	1457.70 ± 30.00[a]	74.75 ± 0.11[a]	804.43 ± 1.44[abc]	653.70 ± 31.50[ab]	1796.00 ± 46.80[bc]	338.13 ± 16.57[a]	992.10 ± 48.3[cde]
	60	508.80 ± 1.04[a]	1547.00 ± 93.50[a]	71.17 ± 3.30[a]	984.00 ± 46.80[a]	563.40 ± 47.20[ab]	2176.70 ± 73.30[ab]	629.10 ± 20.30[ab]	1192.70 ± 26.60[abcd]
	90	482.27 ± 10.28[bcde]	1439.40 ± 72.60[ab]	71.68 ± 1.27[a]	821.10 ± 80.80[abc]	618.40 ± 41.40[ab]	2028.00 ± 112.30[ab]	555.00 ± 111.70[ab]	1206.80 ± 55.10[abc]
350	30	500.43 ± 6.00[ab]	1354.30 ± 60.00[abc]	72.89 ± 0.47[a]	859.70 ± 19.60[abc]	494.80 ± 40.90[ab]	1809.30 ± 72.20[bc]	454.83 ± 11.55[a]	949.60 ± 52.50[cde]
	60	504.53 ± 2.02[a]	1413.00 ± 218.00[ab]	65.77 ± 13.64[a]	916.30 ± 116.60[ab]	496.40 ± 101.00[ab]	2051.00 ± 361.00[ab]	638.20 ± 143.10[ab]	1135.00 ± 244.00[bcd]
	90	495.30 ± 2.08[abc]	1502.00 ± 20.80[a]	73.09 ± 0.45[a]	969.90 ± 119.70[a]	601.40 ± 98.90[ab]	2193.00 ± 365.00[ab]	691.00 ± 345.00[ab]	1292.00 ± 246.00[abc]
400	30	491.57 ± 6.70[abcd]	1591.00 ± 347.00[a]	69.87 ± 8.42[a]	801.60 ± 19.30[abc]	789.00 ± 367.00[a]	2269.00 ± 205.00[ab]	678.30 ± 142.20[ab]	1467.00 ± 225.00[ab]
	60	475.23 ± 13.91[de]	1714.00 ± 41.60[a]	73.89 ± 0.81[a]	761.50 ± 45.00[bcd]	573.50 ± 91.30[ab]	2224.70 ± 37.00[ab]	683.53 ± 10.33[ab]	1399.90 ± 155.10[ab]
	90	476.90 ± 3.74[cde]	1544.00 ± 22.50[a]	71.08 ± 0.09[a]	848.97 ± 2.89[abc]	684.23 ± 2.48[a]	2418.70 ± 17.90[a]	884.50 ± 23.60[b]	1586.33 ± 9.24[a]

[a] Values are represented as mean ± SD. Values in the same column with different lowercase letters differed significantly as determined by ANOVA followed by Tukey's test ($p < 0.01$).
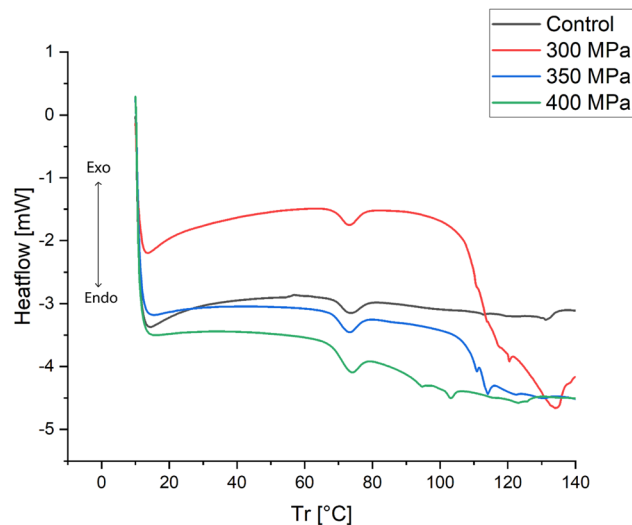

Fig. 2 Thermogram of untreated and high-pressure processed pearl millet (treatment time = 90 min). All the data are presented within the manuscript itself.

2.10. Statistical analysis

All analyses were carried out in triplicate, and the results are reported as mean ± S.D. The statistical analysis was conducted using Minitab version 19.1. Tukey's multiple range test was used to analyse the difference in the means at a significance level of 5% with a one-way ANOVA (variance analysis) among the treatments.

3. Results and discussion

3.1. Effect of high-pressure processing on the colour of pearl millets

Physical qualities, such as color, are an important attribute to be considered for the consumer acceptance of processed food. The color of high-pressure processed pearl millets is illustrated in Table 1. The L^* value representing the lightness of the control millet sample was 53.80 ± 0.26, whereas the L^* values of the treated samples ranged from 48.32 ± 1.00 to 50.74 ± 0.45. The a^* and b^* values of the control were 2.62 ± 0.12 and 21.72 ± 0.14, respectively. The pressure level had less effect on the color of the millet samples ($p > 0.01$), but the treatment time had a significant effect on the color ($p < 0.01$) (Table 1). A longer treatment time (90 min) at 300 MPa resulted in higher color intensity differences (ΔE). High-pressure soaking would have probably caused the leaching of the color pigments into the water. Due to high-pressure processing, the starch molecule's hydrolysis could also cause the color shift into smaller sugar fragments and sugar acids.[23]

3.2. Firmness of pearl millets with a high-pressure processing

The firmness of millets analyzed using a texture analyzer is shown in Table 2. The millet samples showed significantly different firmness values ($p < 0.01$). Though soaking could

Table 6 Thermal properties of untreated and high-pressure processed pearl millet flour

Treatment (MPa)	Treatment time (min)	Gelatinization							Glass transition		
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		Integral (mJ)	Normalized ($J\ g^{-1}$)	Onset temperature [T_o (°C)]	Peak temperature [T_p (°C)]	Endset temperature [T_c (°C)]	Temperature range [T_R (°C)]		Onset (°C)	Midpoint (°C)	Endpoint (°C)
Control	—	−5.67	0.81	69.39	73.38	78.21	8.82		111.67	112.12	112.57
300	90	−6.46	−0.81	69.23	73.1	77.44	8.21		119.75	120.04	120.12
350	90	−8.31	−0.125	68.41	73.04	77.56	9.15		111.41	112.35	113.65
400	90	−10.32	−1.34	68.38	73.68	77.95	9.57		101.33	102.54	103.05

soften the millets due to increased moisture content, drying produced increased firmness. High pressure soaking of pearl millets showed increased firmness compared to the control samples. The firmness of the treated pearl millets ranged from 3742 ± 1044 g to $21\,355 \pm 5469$ g (Table 2). The treated millet samples showed higher firmness values, which could be attributed to the millet starch aggregating into lumps.[24] High firmness and high structural strength make it suitable for use as a fat replacer and for the development of low-fat bakery products.[27]

3.3. Impact of high-pressure processing on the nutritional quality of pearl millets

High-pressure soaking significantly changed ($p \leq 0.01$) the nutritional values of pearl millets, as illustrated in Table 3. With increasing the pressure and soaking time, the moisture content of the soaked millets varied significantly ($p < 0.01$). The water uptake of treated millets increased, which may be caused by the rupture of outer cell walls with a high-pressure treatment A similar result was reported wherein the final moisture content of the brown rice soaked at higher pressures was high, irrespective of the time.[22] The protein content of the control sample was $10.51 \pm 1.16\%$, whereas the treated samples had a protein content of $10.21 \pm 0.25\%$ to $12.84 \pm 0.25\%$ (Table 3). The fat content of the control sample and soaked control was $4.46 \pm 0.76\%$ and $5.22 \pm 0.62\%$, respectively. No significant effect was observed in the fat content percentage of the pearl millets on treatment, which ranged from $4.49 \pm 0.10\%$ to $5.52 \pm 0.43\%$. The untreated sample showed the highest ash percentage of $1.43 \pm 0.03\%$, while the ash content of the treated samples varied from $0.92 \pm 0.01\%$ to $1.41 \pm 0.12\%$. The % carbohydrate content calculated on a difference basis varied from $50.71 \pm 0.35\%$ to $59.93 \pm 3.14\%$. There was a significant difference in the % carbohydrate content after various high-pressure processing, however, the treatment times did not show any significant differences. The decrease in carbohydrates can be due to soluble starches leaching out during the soaking of pearl millets, which can be attributed to the softening effect of soaking in water under high pressure for longer times.[28]

3.4. Effect of high-pressure processing on phytate

Table 4 shows the phytate content of the control, soaked control, and pearl millets with high-pressure soaking for different times (30, 60, and 90 min). The phytate content in the

control samples was 172.13 ± 7.50 mg/100 g. About 17% reduction of phytate content was observed in pearl millets soaked in water for 90 min without high pressure. The phytate content of millet samples significantly decreased ($p < 0.05$) for all the pressure levels and soaking times (Table 4). Up to 80.61% reduction of phytate in treated samples was observed compared to the control. This could be attributed to the enhancement of the phytase enzymatic activity during the soaking of millets under a high pressure. Soaking under a high pressure increases the water inhibition rate in the pearl millet, which leads to improved phytase activity.[29] High pressure processing can also increase enzyme activity by releasing membrane-bound enzymes and by modifying protein shape and the active site to improve substrate binding.[30] An increase in phytic acid–cation complexes' solubility may have caused phytate degradation in pearl millets during the high-pressure treatment similar to those resulting in rice and its bran, as reported by previous researchers.[31] The treatment of foxtail millets at 400 MPa and 40 °C had the highest reduction of phytic acid (67.87%) and the decrease is ascribed to the heat-liability and water-soluble nature of phytate, which breaks down at a higher pressure and leaches into the water.[32] High pressure alters the phytase structure, mainly on quaternary and tertiary ones, which leads to exposure or entrapment of the active site. Additionally, high-pressure processing is considered to increase enzyme reactivity under pressure, when both the enzyme and substrate are processed together.[33] However, the enzymatic activity at different pressure levels was not evaluated in this study. A moderate pressure accelerates the enzyme activity while higher pressure causes protein denaturation and inactivation of enzymes.[34] Hence, the pressure level was not made to exceed more than 400 MPa in the experiments.

3.5. Effect of high-pressure soaking on the availability of free iron

The effect of high-pressure soaking for 90 min at three different pressure levels (300, 350, and 400 MPa) on the iron content of pearl millet samples is shown in Fig. 1. The control sample had an iron content of 127.73 mg kg^{-1}. The soaking and high-pressure processing of the pearl millet showed an increasing trend in the free iron content. The improved free iron content is attributed to the reduction of phytate content in the treatment of the samples, which increases the iron bioavailability.[35] The phytate bonds with minerals are broken down due to soaking at high pressure, and the degradation of phytate to its derivative

forms increases the bioavailability of minerals. The free iron content increased to 579.24 mg kg^{-1} when pearl millets were soaked at 350 MPa for 90 min. However, the sample treated at 400 MPa for 90 min showed a decrease in the free iron content. This could be caused by the leaching of the iron content into the water at very high-pressure levels.[36] Another reason for this reduction may be the possibility of the iron forming complexes with other compounds during high-pressure processing.[37] This study primarily focused on the overall trend of iron content changes under high-pressure soaking but did not delve into the specific compounds or mechanisms responsible for iron loss at 400 MPa. To address this limitation, future research can include a detailed analysis of the soaking to quantify potential iron leaching and identify any iron-binding compounds formed under high pressure. Techniques such as ICP-MS for mineral profiling and FTIR or XRD for complex identification could provide a clearer understanding of these interactions. Understanding the threshold at which pressure-induced iron loss occurs is critical for optimizing processing parameters to maximize nutritional benefits while minimizing losses. Further experiments can also be done to study the mineral contents in the processed millets.

3.6. Effect of high-pressure processing on the techno-functional properties of pearl millets

The molecular conformations, intricate interactions between structures, and the physicochemical qualities of the flour components are all reflected in the flour's hydration and functional properties. Hence, it is crucial to investigate the impact of food processing on the techno-functional properties of the treated millets.

3.6.1. Water absorption capacity (WAC), oil absorption capacity (OAC), and water solubility. The water absorption capacity and oil absorption capacity demonstrate the behavior of the millet flour components in the presence of water or oil, describing the flour's consistency and flavor retention capabilities. High-pressure processing can cause alterations in the secondary protein structure and produce cross-linkages in starch. The WAC and OAC of a sample are influenced by the impact of starch and protein structural modifications.[38]

The quantity of water absorbed and held by the sample after exposure is known as water absorption capacity. It is a significant characteristic in formulations of food, especially those dealing with dough handling.[39] The WAC of the control was $226.10 \pm 4.86\%$, while the treated samples had a range of WAC from $211.59 \pm 3.61\%$ to $253.45 \pm 8.15\%$, as shown in Table 2. The maximum WAC was observed in the sample treated for 90 min at the pressure of 300 MPa, which was a 12.1% increase compared with the control sample. The enhancement of WAC at high pressure can be attributed to the breaking down of non-covalent bonds of the proteins, causing the exposure of more hydrophilic domains of various proteins attributed to the increase in water absorption. The increase in WAC can also reflect its high affinity to water due to starch damage during high-pressure processing, which creates a porous structure that absorbs water *via* capillary action.[40]

Under higher treatment settings, the OAC of the control sample which was $204.51 \pm 2.86\%$ increased to $216.74 \pm 1.99\%$, or approximately a 5.98% increase. Though the OAC did not show much increase when treated at 300 MPa, it improved on higher pressure and time treatments (Table 2). High OAC reflects more polar amino acids, favouring oil affinity in the samples. Treated samples show improved OAC due to proteins entrapping oil in between the proteins' non-polar side chains.[41] WAC and OAC are crucial characteristics of the flour that influence the consistency of many applications in baking industries.[42]

The control millet flour had water solubility of 0.05 ± 0.02 g g^{-1} and decreased to 0.03 ± 0.01 g g^{-1} for high-pressure treatments (Table 2). The water solubility of the treated millet flour decreased with increasing the pressure level and treatment time. The decrease in water solubility can be attributed to starch degrading to simple sugars such as dextrin, oligosaccharides, and other fermentable sugars during high-pressure processing.[43]

3.6.2. Pasting properties. The pasting properties of millet flour describe its cooking quality, the texture profile of the flour, and the products developed from it during processing. Pasting properties include peak time, peak viscosity, pasting temperature, holding strength, breakdown viscosity, final viscosity, and setback from peak and trough. The concentration of starch directly impacts the pasting properties of millet flour. The viscosity of the flour is influenced by starch swelling, gelatinization, protein denaturation, gluten content, *etc.* Table 5 shows the pasting properties of the control and pearl millets soaked without and with high-pressure. High-pressure treatment had a significant effect on the pasting properties of millets ($p < 0.01$). The maximum viscosity achieved by starch when heated in water is peak viscosity, reflecting the WAC of starch granules. Damage to the structure of starch granules allows them to swell to a greater extent, causing a high peak viscosity.[44] The high-pressure treatment improved the flour's pasting viscosity (Table 5). Treatment at 300 MPa resulted in flour with a higher pasting temperature. This implies that the carbohydrate components of pearl millet will not break down until a higher gelling temperature is attained.[45] However, higher pressure treatment for longer times showed lesser values of pasting temperature. The degree to which swollen starch granules are broken down by mechanical agitation and heating is measured by breakdown viscosity. The higher breakdown viscosity shows that the starch granules during the cooking process cannot endure heat and have a reduced resilience to shear stress.[46] The retrogradation and reorganization of amylose linkages between the starch molecules caused elevation in the final viscosity of the sample on increased pressure levels and treatment time. This may be because the hydrogen bonding breaks down when starch modification allows for a considerable quantity of water to be incorporated, resulting in a high final viscosity.[47] A rise in setback values from the trough indicates a declining digestibility of dough. In contrast, the reduction in setback from the peak during paste cooling implies it has a lower tendency to retrograde and less syneresis. In most industrial applications, the capacity of starchy paste to thicken on cooling or become

thin following subsequent heating is desired.[48] Pasting qualities are also affected by changes in amino acid profiles in the sample.[49]

3.6.3. Thermal analysis. The thermal properties of the millet flour were analyzed using a differential scanning calorimeter (DSC). The heat absorbed (endothermic process) or liberated (exothermic process) affects the physical state and crystalline modifications of starch.[50] The thermograms of the control and high-pressure processed pearl millets for 90 min are depicted in Fig. 2. The first peak in the DSC thermogram at lower temperatures (70–80 °C) indicates the gelatinization of starch, while the second peak at high temperatures indicates the amylose–lipid complex.[51] Gelatinization temperatures represent the stability of the starch molecule structure. The onset T_o temperature, peak temperature T_p, and completion temperature T_c of the control samples were 69.39 °C, 73.38 °C, and 78.21 °C, respectively. In comparison to the control sample, the high-pressure treated samples had lower gelatinization temperatures and enthalpies (Fig. 2). High-pressure treatment at 300 MPa resulted in low gelatinization temperature, which may be due to the breakdown of the double helixes in the crystalline and amorphous regions of the granules and the amylose–amylopectin interactions with amylose–amylose chains. Though the gelatinization temperature range of high-pressure processed samples at 300 MPa was less, samples processed at 350 MPa and 400 MPa resulted in a higher range than that of the untreated samples. Pressure treatment gradually causes gelatinization, as reported in previous studies.[52] The DSC measures T_o, T_p, and ΔH indicate the crystalline stability and crystallinity of amylopectin and loss of double helix order. Gelatinization of starch granules includes hydration, swelling, and release of granular amylose. The shape and crystal structure are lost during this process. The granule structure is maintained in the high-pressure process due to the limited swelling of starch granules. A similar trend in the results was seen in a previous study.[53] These also relate to the firmness results and other techno-functional properties of millets observed. The DSC parameters of the control and high-pressure treated samples are illustrated in Table 6. The glass transition of pearl millets treated at 300 MPa showed the highest onset temperature value, followed by the untreated sample, samples treated at 350 MPa, and 400 MPa. These changes agreed with those described in the high-pressure treatment of rice starch,[54] brown rice,[55] and foxtail millet.[32] This agreed with the findings reported as the glass transition temperature T_g values decreased with an increase in the moisture content.[56]

4. Conclusion

The pressure levels and treatment time significantly impacted the pearl millet's characteristics. Soaking pearl millets at high pressure is an efficient and green technology for lowering phytate contents and increasing the free iron. There is an 80.61% reduction in phytate content after soaking at 400 MPa for 90 min and the highest free iron content was obtained with soaking for 90 min at a pressure of 350 MPa. The color, firmness, water absorption, oil absorption, and water solubility of treated pearl millet samples exhibited a statistically significant difference compared to the control ($p \leq 0.05$). High pressure resulted in improved techno-functional properties of the pearl millets, making it easier to use in product formulations and providing diverse uses. High-pressure treatment also significantly influenced the pasting and thermal properties of the millet starch, which can be optimized to get the desired consistency to prepare new convenient food products.

HPP transfers heat through convection, conduction, or radiation, resulting in slower heat transfer rates and longer processing times. However, it eliminates the need for additional energy to generate heat, making it an energy-efficient process by applying isotactic pressure using a specific amount of energy. This reduction in heating requirements further highlights the potential for HPP to be a sustainable and cost-effective technology in food processing. In conclusion, high-pressure soaking not only enhances the mineral bioavailability of pearl millet but also improves its functional properties, positioning it as a valuable ingredient for a wide range of food products, including fortified foods, plant-based alternatives, and convenience foods. Future research should focus on understanding the functional behaviour of HPP-treated millet in different food systems, evaluating its long-term stability, and exploring its scalability for industrial applications. Additionally, studies on the interaction of HPP with other grains and its potential to reduce other anti-nutritional factors would provide further insight into its broader applications in the food industry.

Data availability

All the data are presented within the manuscript itself.

Conflicts of interest

Authors do not have any known financial or non-financial interests directly or indirectly related to the work submitted for publication.

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