Canrea Journal: Food Technology, Nutritions, and Culinary, 2025; 8 (1): 184-200 OPEN ACCESS

e-ISSN : 2621-9468

Type of Paper (Article)

Chilli paste processing using high-pressure and conventional approaches

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Abstract

This study presented a comparative analysis between chilli paste processed using high-pressure processing (HPP) and traditional thermal treatment (TT) to assess their influence on bioactive attributes, physicochemical properties, and safety. Five distinct processing parameters were examined: untreated (control), HPP at 200, 400, and 600 MegaPascals (MPa), respectively, treated for 6 minutes, and TT at 80°C for 10 minutes. Significant differences ($p<0.05$) were observed between HPP and TT across most physicochemical properties and all bioactive constituents, barring the hue angle (h), Aw, and pH. HPP-treated samples closely resembled the control in terms of physicochemical and bioactive characteristics. Notably, HPP at 400 MPa yielded the highest concentrations of ascorbic acid (AA), cumulative phenolic compounds (CPC), and radical scavenging activity (RSA). Conversely, HPP at 600 MPa exhibited the most robust pungency properties, as reflected by capsaicin (CAP), dihydrocapsaicin (DHC), and Scoville heat units (SHU). TT samples had the most microbial inactivation, although a 5-log reduction level was not reached. Bacillus cereus (ATCC 14579) count, total plate count (TPC), and yeast and mould count (YMC) reductions are intensified at higher HPP pressures. HPP applied at 400-600 MPa for 6 minutes was deemed superior in maintaining quality compared to TT, while also retaining safety levels. In summary, HPP proves to be a proficient technique for processing chilli paste, with minimal disruption to its physicochemical and bioactive attributes. HPP at 400-600 MPa for 6 minutes is a promising alternative, offering both quality preservation and safety advantages over traditional methods.

Article History
Received March 14, 2024
Accepted June 4, 2025
Published July 30, 2025

Keywords
Chilli Paste, High-Pressure Processing, Thermal Pasteurisation, Capsaicin, Dihydrocapsaicin, Quality Preservation.

1. Introduction

Chilli (*Capsicum annuum L.*) consumes at least 65.46 million kg annually in Malaysia (1). Chilli contains nutrients such as antioxidants, vitamins, and minerals (2). Chilli can be consumed raw in the form of solids and powder or turned into a paste. Traditional preparation of chili paste involves size reduction, mixing, and heating. While exposure to heat enhances safety, it can also affect paste quality, often necessitating the excessive use of

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ISSN 2621-9468 online, DOI 10.20956/canrea.v8i1.1279

preservatives and colorants, (3–5). Moreover, one of the common bacteria causing spoilage in fresh chilli and chilli paste is *B. cereus*, which can survive inadequate thermal treatment. To address these concerns, the inactivation efficiency of *Bacillus cereus* with American Type Culture Collection (ATCC) number; 14579 was evaluated using both thermal and non-thermal methods, such as irradiation and high-pressure processing (HPP). Non-thermal techniques have been shown to preserve quality without compromising safety (6–8), resulting in a fresh-like volatile profile, as well as maintaining phenolic and capsaicin contents with lower microbial counts (9,10).

However, existing studies have primarily focused on fresh chili paste, with limited research addressing the application of HPP to dried chili pastes. This represents a significant gap in current knowledge, considering that dried chillies are widely used in paste production and are particularly susceptible to *B. cereus*, especially under suboptimal environmental and post-harvest conditions (11). This study aims to evaluate the efficacy of high-pressure processing (HPP) and thermal pasteurization (TT) methods in enhancing the quality of chili paste and reducing the microbial presence, focusing specifically on chili paste prepared from dried chilies.

2. Materials and Methods

2.1. Chemicals

Bacillus cereus selective agar (BCA) (Oxoid, Hampshire, England), peptone water (Oxoid, Hampshire, England) nutrient broth (Oxoid, Hampshire, England), plate count agar (PCA) (Oxoid, Hampshire, England), Potato Dextrose Agar (Oxoid, Hampshire, England), Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), ethanol (John Kollin Corporation, Edinburgh, UK), 2.6-dicholorphenolindophenol (DCPIP), 2,2-diphenyl-1-picrylhydrazyl (DCPIP), Acetonitrile, Capsaicin and Dihydrocapsacin standard, Acetic Acid.

2.2. Raw Materials

Dried chilli selection was performed according to the Federal Agricultural Marketing Authority guideline (12). Chilli variant 668 (*Capsicum annuum L.*) was purchased from a wholesaler based on appearance, size, and color, storing them at 4 °C in a sealed plastic bag.

2.3. Preparation of Chilli Paste

Chilli paste was prepared at a 1:3 (v/w) water-to-chilli ratio following preliminary laboratory tests to optimize mixing, enhance filling, and ensure treatment consistency. Initial processing involved stems removal and deseeding, which was accomplished using a Thermomix® TM6® food processor at turbo mode (10,700 rpm) for one second, repeated three times, followed by manual deseeding through sieving (mesh 0.8 cm) using the Thermomix® varoma system. =The dried chilies were weighed and manually rinsed in a metal strainer to remove seeds and any residual material.

2.4. Experimental Design

Chilli pastes were filled into polyethylene terephthalate (PET) bottles and divided into three treatments groups. Each bottle contained approximately 25 g of chilli paste. For the thermally processed pastes (without inoculation), the samples were bottled only after

exposure to thermal treatment. For inoculated pastes, the bottles were vacuum-sealed using Vacmaster (Vacmaster VP 545, Kansas, United States) in double bags to prevent leakage.

2.5. Pasteurization of Chilli Pastes

The bottles were processed using a commercial-scale high-pressure processing (HPP) system (Wave 6000/55 Hiperbaric, Burgos, Spain) at 200, 400, and 600 MPa for 6 min with a pressure come-up time of approximately 325 MPa/min and rapid depressurization (2s) to 0.1 MPa. A specially designed pressure vessel filled with distilled water was used for indirect pressurization, maintaining the processing temperature at room temperature (RT) (24 ± 2 °C).

Chilli pastes without *B. cereus* (ATCC 14579) inoculation underwent thermal treatment using a Roboqbo QBO 15-4 (Roboqbo, Bentivoglio, Italy) under vacuum conditions (-900 mbar) at 80°C for 10 min at a speed of 500 rpm. A water bath system (Memmert, Büchenbach, Germany) was used to heat the chilli pastes to 80 ± 1.5°C for the inoculated pastes for 10 min. The Roboqbo QBO 15-4 system applies vacuum concentration to efficiently remove moisture from the product.

2.6. Preparation of B. Cereus Vegetative Cells

The preparation of *B. cereus* (ATCC 14579) vegetative cells was performed according to Jeon et al. (13) with modification of the culture broth. A stock culture containing 108 CFU/mL of *B. cereus* (ATCC 14579) was preserved at −20 ± 2 °C with 30% glycerol. The cultivation of Bacterial cells was carried out on BCA plates at 35 ± 2°C for 24 h. After incubation, a single colony was inoculated into the nutrient broth at 35 ± 2°C for 24 h to obtain microbial concentration of 108 CFU/mL. The concentration of bacteria was determined by measuring the optical density (OD) of the culture broth using a visible spectrophotometer Genesys 20 at 600nm (Thermo Fisher Scientific, Waltham, Massachusetts). The OD600 value was standardized at 0.5±0.05, which corresponded to 107–108 CFU/mL (13). The vegetative cells in the culture broth were validated by PCA.

2.7. Inoculation of B. cereus (ATCC 14579) Vegetative Cells

Chilli pastes (25 g) were inoculated with 1 mL of nutrient broth containing *B. cereus* (ATCC 14579) vegetative cells yielding an initial concentration of 10^5–10^6 CFU/g. Broth mixing was performed gently usinga sterilized stainless-steel spoon for uniform cell distribution (13). Non-inoculated chilli paste served as a negative control to assess the effect of high pressure on *B. cereus* (ATCC 14579) in the paste. This study focuses on bacterial contamination, therefore, the specific target organism *B. cereus* is inoculated into chilli paste while assessing Total Plate Count (TPC) and Yeast and Mold Count (YMC) using indigenous microbes.

2.8. pH Value and Water Activity (Aw)

Chilli paste pH was measured in triplicate using a pH meter (3505 Jenway, Staffordshire, United Kingdom) following AOAC 943.02 analysis principle (14). Prior to recording, a 1:10 (w/v) dilution with distilled water was prepared in room temperature (RT) 24 ± 2 °C, and the pH sensor was rinsed with distilled water and dried before each analysis. Water activity (Aw) was determined in triplicate using an Aw analyzer (AquaLab model series 3TE, Decagon, USA) by placing the sample in the Aqualab chamber to record Aw readings.

2.9. Colour

Chilli paste colour profile was analyzed using a chromameter (CR-400, Konica Minolta, Tokyo, Japan) in triplication. Colorimetric diagrams were used to decipher the values for brightness (L*), greenness (a*), and blueness (b*) while the hue angle (hº) and chroma (C*) were calculated using equations from (15):

$$(hue\ angle) = \tan^{-1}\left(\frac{a^*}{b^*}\right)$$

$$C^*(Chroma\ Value) = \sqrt{a^{*2} + b^{*2}}$$

where;

a^* = Redness or Greenness and b^* = Yellowness or Blueness

2.10. Texture Profile

A texture profile analyzer (TA-XT2i, Stable Microsystem, London, UK) was used to assess chilli paste's firmness, consistency, cohesiveness, and viscosity index. The extrusion parameters were based on Nadia Sarina et al. (16), Sobhi et al. (17), and Cheok et al. (10) with slight adjustments. The samples were placed in a 75%-filled standard-sized back extrusion container with a 1-inch (2.54 cm) carbon ball-type probe centred over the sample. After each test, the probe was calibrated 30 mm above the surface of the sample to compare cohesiveness and cohesion. The extrusion container was filled by tilting it slightly to reduce the air pockets during testing.

2.11. Microbial Count

The treated chilli paste, 25 g per batch, was individually homogenized in 225 mL of sterile peptone water for 1 min using a stomacher (Lab Blender 400, Gemini BV, Overijssel, Netherlands) resulting in a 10−1 dilution of *B. cereus* (ATCC 14579). Following homogenization, chilli paste samples were serially diluted a 10-fold in 0.1% peptone water until reaching a 10-5 dilution factor. Duplicate 0.1 mL of each dilution was spread-plated onto *Bacillus cereus* selective agar (BCA) for selective growth of B. *cereus* (ATCC 14579). After 48 hours of aerobic incubation at 35 ± 2°C, colonies of viable vegetative cells were counted with a colony counter (Galaxy 230, Wiggens, Straubenhardt, Germany) and the population of B. cereus (ATCC 14579) vegetative cells was expressed as log10 CFU/g. the logarithmic reduction of *B. cereus* (ATCC 14579) cells in chilli paste was calculated by subtracting the count in treated samples from the initial count before treatment.

Viable microorganisms other than *B*. *cereus* (ATCC 14579) were enumerated using total plate count (TPC) on plate count agar (PCA) and yeast and mold count (YMC) (YMC) on potato dextrose agar (PDA). Serial dilutions of untreated and treated samples were prepared by mixing 25 g of sample with 225 mL of sterile peptone water. For TPC, 1 mL was poured onto the plate, and each dilution 0.1 mL of each dilution was plated onto duplicate plates and spread evenly with a sterile plate spreader to determine the YMC. TPC was determined using PCA, whereas YMC was assessed using PDA. Plates were incubated aerobically at 37 ± 1°C for 48 ± 2 hours for TPC and 30 ± 1°C for five days for YMC. The results are expressed as log colony-forming units (log 10 CFU/g) for each sample (18). Microbial counts in treated (N_1) and control (N0) chili paste samples were compared to compute logarithmic TPC and YMC reductions.

Therefore, logarithmic reduction can be determined using the following equation, log10 (N_1/N^0).

2.12. Ascorbic Acid Content

Spectrophotometer measurements were used for ascorbic acid detection in chilli paste due to interference from the thick orange-red colour pigment during titration. The chilli pastes reagent preparation f was performed according the ratio (19). The ascorbic acid content was measured using spectrophotometric analysis of Sayin and Arslan (20). At RT (24 ± 2 °C), 200 mg paste was extracted with 10 mL 1% metaphosphoric acid and filtered through Whatman No. 4 filter paper. The filtrate was combined with 9 mL of 2.6 - dichlorophenolindophenol (DCPIP). The absorbance was measured within 30 min at 515 nm against a blank due to continual ascorbic acid deterioration. Ascorbic acid was estimated using at calibration curve of pure L-ascorbic acid (0.020-0.12 mg/mL). Triplicate experiments yielded mean values ± standard deviations of ascorbic acid in mg/g extract.

2.13. Extraction of Chilli Paste

One gram of chilli paste was mixed with 50% ethanol and extracted with a stirrer (LMS Co., Tokyo, Japan) at RT (24 ± 2℃) for 30 min. The sample was centrifuged at 4200 × g for 5 min to collect the supernatant.

2.14. Cumulative Phenolic Content (CPC)

CPC was evaluated following the method of Fu et al. (21) with modification. 0.2 mL of the supernatant was mixed with 2.5 mL of 1:10 diluted Folin-Ciocalteu reagent, and 4 minutes later, 2 mL of 75 g/L sodium carbonate was added. After 120 min of incubation in total darkness at RT at 24 ± 2 °C, the mixture was evaluated using a UV-VIS spectrophotometer (Thermo Fisher Scientific, Wisconsin, United States) at 760 nm. The standard curve was measured against Gallic acid equivalents (mg GAE) per 100 g wet paste weight.

2.15. DPPH Radical Scavenging Activity (RSA)

The radical scavenging activity (RSA) was measured using the (2,2-diphenyl-1-picrylhydrazyl), following the method described by Yeng et al. (22) method. A 0.5 mM DPPH reagent was prepared in 80% ethanol and the chilli paste supernatant (0.5) ml) was mixed with 0.3 mL DPPH reagent. After 100 min of incubation in the dark, a UV-vis spectrophotometer (Thermo Fisher Scientific, Wisconsin, US) was used to assess the absorbance of the solution at 517 nm. RSA was measured using the following formula:

$$DPPH\ Radical\ Scavening\ Activity\ (\%) = \left[\frac{(a0 - as)}{a0}\right] \times 100\ \%$$

Where; a0 = absorbance of a blank and as = absorbance of a tested sample

2.16. Capsaicinoids Content and Scoville Heat Unit

High-Pressure Liquid Chromatography (Shimadzu LC-6, Kyoto, Japan) was used for the extraction and measurement of capsaicinoid content followed by Cheok et al. (10). A 10 g of Capsaicin (CAP) was homogenised with 40 mL HPLC-grade acetonitrile using a shaker

(M65820, Thermolyne, USA) at 300 rpmfor 10 h at 25°C. The extract was filtered to obtain a clear supernatant. A 20-µl portion was injected into Thermo Hypersil Gold C18 (2504.6 mm, Thermo Scientific, USA). LCL solution software (Version 1.21 SP1, Kyoto, Japan) was used in the HPLC system equipped with A fluorescence detector (Shimadzu LC-6A, Kyoto, Japan). The isocratic eluent was acetonitrile, deionized water, and acetic acid (100:100:1, v/v/v) at 1 mL/min at RT 24 ± 2 °C. The detector's excitation and emission wavelengths were set to 270 and 330 nm. Capsaicin and dihydrocapsaicin were expressed in mg/100 g dry mass. HPLC analysis determines capsaicinoid values which dictates the Scoville heat unit (SHU). Using sample dry weight, both alkaloids were quantified in ppmH (23). The ASTA (24) formula was used to calculate capsaicinoids as describedby Usman et al. (25):

$$ppmh: \frac{[peak\ area\ (capsaicin) + 0.82 \times peak\ area\ (Dihydrocapsaicin)] \times ppm\ standard\ \times ml\ acetonitrile}{peak\ area\ (standard) \times g\ sample}$$

The conversion to Scoville Heat Units was accomplished by multiplying ppmH by 15 (25).

2.17. Statistical Analysis

The reported results were analyzed using the MINITAB software (Version 20, Pennsylvania, USA). One-way analysis of variance (ANOVA) was used to identify significant differences between the results, and with Tukey's test was used to separate means at a significance level of 0.05.

3. Materials and Methods

3.1. pH value and Water Activity (Aw)

Both processed and unprocessed sample pH and Aw are shown in Table 1. Regardless of treatment, all samples pH (5.0 – 5.05) and Aw (0.930–0.943) were insignificant differences ($p > 0.05$). The experimental results were in concomitance with others. Hurtado et al. (26) found insubstantial changes in pH values of both thermal-treated (85°C for seven min.) and HPP-treated (350 MPa for five min) red fruit-based smoothies. In addition, both Li et al. (27) coriander paste and Nath et al. (28) fermented minced pepper pH and Aw were not significantly altered upon pressurization. The result highlights pH and Aw stability under HPP and thermal.

Table 1.The value of pH and A_W of raw and pasteurised chilli pastes.

Analysis	Untreated (Control)	High-pressure (6 min)			Thermal (80°C 10 min)
		200 MPa	400 MPa	600 MPa	
pH	5.032±0.37[a]	5.045±0.24[a]	5.035±0.25[a]	5.054±0.21[a]	5.001±0.30[a]
Water activity	0.940±0.04[a]	0.943±0.0 [a]	0.942±0.03[a]	0.941±0.03[a]	0.930±0.03[a]

[a] Indicates no significant difference ($p > 0.05$)

3.2. Colour

Chilli paste colour, measured with (CR-400, Konica Minolta, Tokyo, Japan), was expressed using five colour parameters: brightness/lightness (L*), red/greenness (a*), yellow/blueness (b*), hue angle (h), and chromaticity (C*) (Table 2).

Table 2. Colour of raw and pasteurised chilli pastes.

Value	Untreated (Control)	High-pressure (6 min)			Thermal (80°C 10 min)
		200 MPa	400 MPa	600 MPa	
*L**	36.97 ± 0.08[a]	37.12 ± 0.07[a]	37.11 ± 0.12[a]	37.20 ± 0.10[a]	36.13 ± 0.36[b]
*a**	23.81 ± 0.01[a]	24.25 ± 0.12[a]	23.92 ± 0.04[a]	23.93 ± 0.26[a]	22.64 ± 0.29[b]
*b**	11.90 ± 0.69[ab]	12.65 ± 0.12[a]	12.44 ± 0.02[a]	12.52 ± 0.12[a]	11.50 ± 0.29[b]
h	26.55 ± 1.35[a]	27.55 ± 0.11[a]	27.48 ± 0.02[a]	27.61 ± 0.03[a]	26.93 ± 0.32[a]
C*	26.62 ± 0.30[a]	27.35 ± 0.16[ab]	26.96 ± 0.04[ab]	27.01 ± 0.28[b]	25.40 ± 0.38[c]
Visual					

[a, b, c] Indicates significantly different values ($p < 0.05$) between column results

The HPP treatment did not yield any statistically significant differences ($p > 0.05$) in brightness (L*), red/greenness (a*), yellow/blueness (b*), or hue angle (h), as detailed in Table 2. However, the chromaticity (C*) increased notably at 600 MPa. This observation echoes prior studies, where Nath et al. (28) noted a 10–12% increase in color intensity of HPP-treated coriander paste, while Koh et al. (29) observed heightened yellowness and brightness in fruits, evident through changes in chromaticity and hue angle. The breakdown of fruit and vegetable cell walls under HPP liberates pigments, subtly altering color and underlining the treatment's efficacy in retaining colour.

In contrast, heat is likely to increase the chemical reaction rate such as the Maillard reaction, thus resulting in the darkest (L*), lowest red/greenness (a*), yellow/blueness (b*), and lowes chromaticity (C*). As supported by other findings, the negative effect of heat is also present: Sobhi et al. (9) reported heat darkens (L*) chilli paste (32.62 ± 0.53 – 26.29 ± 0.32), Ismail and Revathi (30) reported significant (L*) decrease ($p < 0.05$) as evaporation time and temperature rise, Nath et al. (28) reported heat reduced coriander paste colour intensity. The findings highlight the role of heat in modifying paste colour by reducing its intensity. Pigments such as keto-carotenoid, violaxanthin, lutein, and β-carotene are susceptible to degradation under heat, thereby influencing changes in the colour of the sample (9,30,31).

3.3. Texture Profile Analysis

The chilli paste texture, pasteurization by firmness, consistency, cohesiveness, and work of cohesion were , assessed using a texture analyser (TA.XT2i, Stable Micro System Texture Analyzer, Surrey, UK) as shown tabulated (Table 3).

Table 3. Texture profile analysis of chilli pastes.

Textural Parameter	Untreated (Control)	High-pressure (6 min)			Thermal (80°C 10 min)
		200 MPa	400 MPa	600 MPa	
Firmness	25.27±0.31^{d}	27.74±1.01^{c}	26.16±0.74^{d}	31.94±0.49^{b}	38.23±0.55^{a}
Consistency	929 ± 27.2^{d}	1027 ± 61.1^{c}	947 ± 31.3^{d}	1159 ± 43.9^{b}	1427±18.39^{a}
Cohesiveness	-7.63±0.50^{a}	-9.99±0.50^{c}	-8.65±0.33^{b}	-14.98±0.87^{d}	-26.77±0.31^{e}
Work of Cohesion	-5.88 ± 1.42^{a}	-12.16±6.11^{ab}	-11.38±8.51^{ab}	-16.34±2.05^{b}	-154.31±3.9^{c}

$^{a, b, c, d, e}$ indicates significantly different values ($p < 0.05$) between column.

The thermally treated pastes exhibited the highest firmness, followed by the HPP-treated samples. Notably, all samples showed significant differences ($p < 0.05$) compared to the control (Table 3). Firmness increased in with HPP pressure levels, suggesting a direct correlation influenced by the effect of HPP on pectin methylesterase (PME), a factor known to enhance fruit and vegetable firmness (32). Moreover, consistency, reflecting material flow, displayed significant disparities from the control ($p < 0.05$) across all samples except for those treated at 400 MPa (Table 3), with thermal treatment yielding the highest consistency. At 400 MPa, partial cell wall rupture occurred, releasing intracellular water and soluble that diluted the matrix. However, the pressure was insufficient to inactivate enzymes or enhance structural integrity, resulting in a lower consistency compared to treatments at 200 and 600 MPa. At 200 MPa, the cell structure remained largely intact, retaining more of the original texture and consistency. In contrast, 600 MPa caused extensive cell disruption and enzyme inactivation, promoting the release of polysaccharides and the formation of a more stable, gel-like matrix with higher consistency. The similarity in consistency between 400 MPa and the untreated control is attributed to the combined effects of mild cell rupture, enzyme activity, and matrix dilution. The thermal treatment yielded the highest consistency. This aligns with findings from a related study where HPP enhanced the water binding capacity and viscosity of tomato puree while mitigating serum separation, a contrast to conventional pasteurization methods (33).

Cohesiveness, indicating the material's grip, significantly varied among all treatment groups ($p < 0.05$), with the control exhibiting the highest cohesion and the thermally treated (TT) sample demonstrating the lowest (Table 3). The impact of HPP on cohesion may vary depending on the specific material involved, as evidenced by differing outcomes in various studies (34,35). HPP's action on cell walls and semi-permeable membranes alters plant tissue structure (36), leading to particle size reduction and smoother texture in chili paste, resulting in a more consistent and pleasant mouthfeel.

Furthermore, cohesion, reflecting material deformation energy, underwent treatment-induced changes, with significant differences ($p < 0.05$) observed in the control, 600 MPa, and thermally treated samples (Table 3). Unlike thermal treatment and electron beam methods, which tend to reduce firmness, consistency, cohesiveness, and cohesion, HPP evenly distributes pressure throughout chili paste, preserving its texture and sensory attributes (10).

3.4. Microbial quality

Chilli paste microbial quality was assessed using logarithmic reduction. Thermal treatment inactivated microorganisms the most, whereas higher pressure in HPP (200–600 MPa) increased inactivation, highlighting HPP's food preservation potential (Table 4). However, all

treatments failed to achiev a 5-log reduction. This could be due to factors such as the resistance of specific microorganism'se.g., spore-forming bacteria), food matrix composition, and HPP parameters.

Table 4. Logarithmic reduction of total plate and yeast and moulds count in heat-treated and high-pressure-treated chilli pastes with and without Bacillus cereus (ATCC 14579) inoculation.

Treatment	Log Reduction (log_{10} CFU/g)		
	Total Plate	Yeast and moulds	*B. cereus* (ATCC 14579)
Thermal (80℃, 10 mins)	1.95	2.04	1.07
High pressure			
200 MPa	1.14	1.08	0.65
400 MPa	1.21	1.29	0.82
600 MPa	1.39	1.58	1.03

The HPP results showed superior TPC reduction compared to the electron beam treatment (8). HPP's microbial inactivation of HPP in chilli paste was supported by Nath et al. (28), who found that 600 MPa of HPP on coriander paste for 5 min decreased microbial count by 2-log-reduction. The target microorganisms were less protected by baroprotection in the Nath et al. (28) coriander paste due to its more significant water activity (Aw). HPP inactivation of vegetative bacteria is greatly affected by food Aw (37,38). Even minor shifts of Aw in HPP can significantly diminish microbial inactivation, as demonstrated by Van Opstal et al. (39), who found that an Aw value of 0.94 effectively shields. We found only an incremental decrease in TPC in chilli paste, both with and without *B. cereus* (ATCC 14579) inoculation due to high solute concentration-induced baroprotection. The sample Aw was 0.94, and the pH was within the recommended pasteurized product range of pH 5 to 4.6.

Thermal treatment resulted in most significant inactivation of *B. cereus* (ATCC 14579), resulting in a 1.07 log10 CFU/g reduction. Thermal treatment negatively affects microorganisms by altering their structural and physiological properties (40). An other study reported that heat treatment at 80 ˚C for 21.6 min resulted in the total elimination of yeast and mould (9). HPP at 200-600 MPa reduced *B. cereus* (ATCC 14579) by 0.65-1.03 log10 CFU/g, with an intensified effect when pressure increased. HPP disrupts microbial cell membranes, causing structural damage and cell death (41). Similarly, the efficacy of HPP on YMC is also highly dependent on the water activity of food (37,38). Furthermore, the effect of HPP on YMC in chilli paste was less pronounced than that reported by Nath et al. (28), who found that YMC was absent in HPP-treated coriander paste. As discussed above, high-pressure treatment can be compromised if a particular water activity level is not achieved. The findings highlight the significance of Aw in HPP treatment. In addition, postharvest contamination and material mishandling may contribute to a higher initial YMC count in untreated chilli paste (3). Unhygienic practices, improper storage, and indigenous microflora may contribute to the overall spike in microorganisms in the samples before treatment.

3.5. Pungecy quality

Capsaicin (CAP) and dihydrocapsaicin (DHC) are potent compounds that gives chilli distinct characteristics. chromatograms of the capsaicin and dihydrocapsaicin peaks are shown (Figure 1).

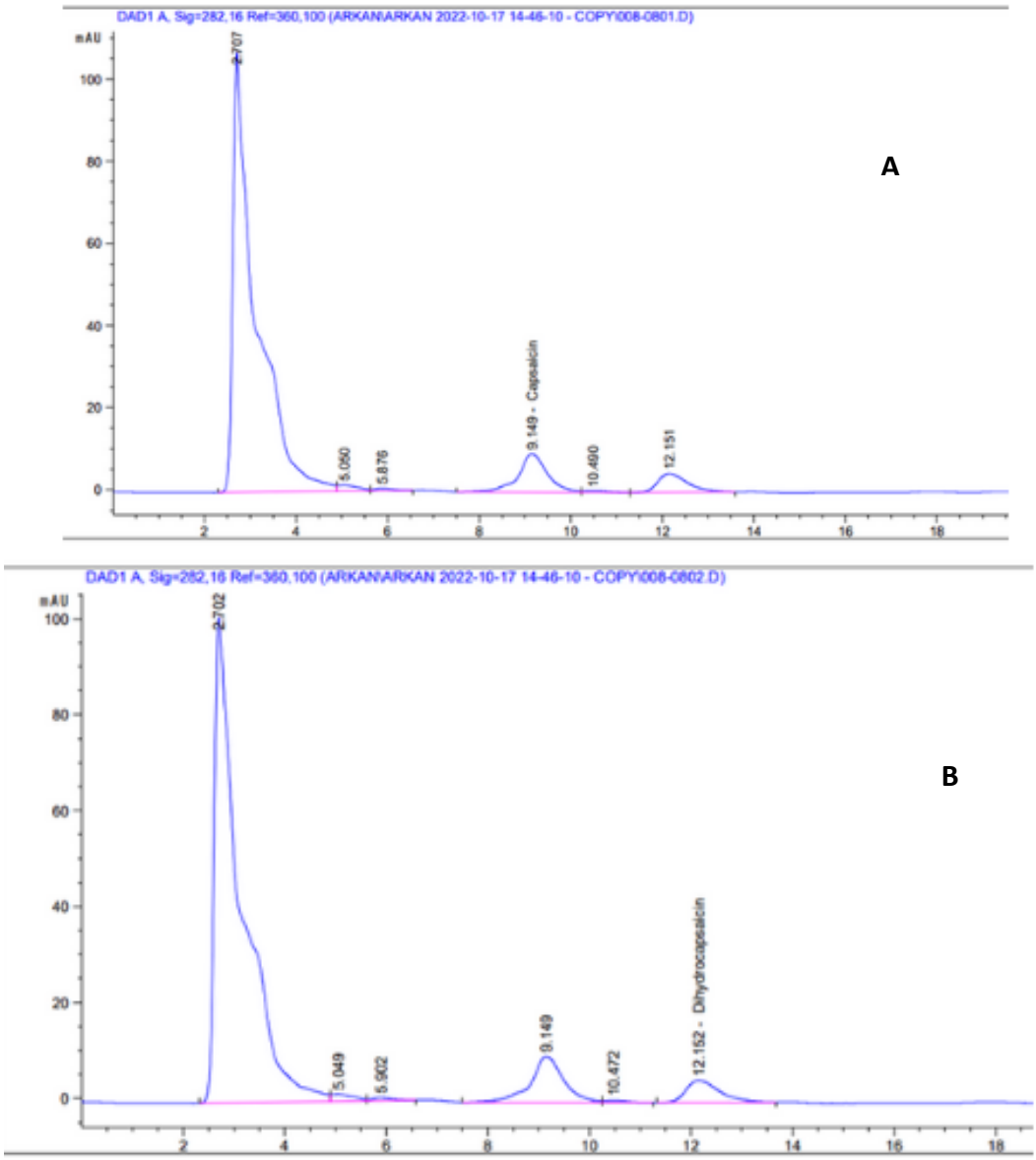

Figure 1. HPLC chromatogram of the capsaicinoids (capsaicin and dihydrocapsaicin) from processed chilli paste using fluorescence detector at 270 nm excitation and 330 nm emission. (A) Capsaicin peak (B) Dihydrocapsaicin peak.

Distinct peaks were clearly discernible for both compounds, serving as crucial data for quantification. As illustrated in Table 5, notable disparities in pungency quality were observed among the untreated, HPP-treated, and thermally treated chili paste samples. The capsaicin content ranged from 31.75 to 42.68 mg/100g across the pastes, while the dihydrocapsaicin content varied from 8.3 to 10.71 mg/100g. Notably, these values far exceed those typically found in Jalapeño peppers, which range from 1.33 mg/g for capsaicin and 0.9625 mg/g for

dihydrocapsaicin, to 0.742–3.197 mg/g for Mexican Jalapeño peppers, and 0.815 mg/g for red Jalapeño peppers (42–44).

Table 5. Capsaicin, dihydrocapsaicin and heat level for chilli pastes.

Parameter measured	Untreated	High pressure (6 min)			Thermal (80°C 10 min)
		200 MPa	400 MPa	600 MPa	
Capsaicin (CAP)	39.78 ± 1.31[a]	40.23 ± 1.05[a]	41.61 ± 0.18[a]	42.68 ± 1.11[a]	31.75 ± 3.08[b]
Dihydrocapsaicin (DHC)	9.93 ± 0.04[b]	10.03 ± 0.03[b]	10.25 ± 0.04[b]	10.71 ± 0.01[a]	8.3 ± 0.24[c]
Scoville heat unit (SHU)	673829 ± 16475[b]	681297 ± 12536[b]	702157 ± 1547[ab]	723739 ± 15849[a]	464697 ± 14260[c]

[a, b, c] Indicates significantly different values ($p < 0.05$) between column.

Thermal treatment affected chilli paste pungency the most ($P < 0.05$), which exhibited the lowest CAP and DHC content, at 31.75 ± 3.08 and 8.3 ± 0.24, respectively. Apichartsangkoon et al. (6) reported a similar trend, whereas capsaicinoids decreased by approximately 50% following thermal treatment. In addition, thermal breakdown and leaching into boiling water can reduce capsaicinoid levels by 28.3%(45). Thermal treatments breaks capsaicin (8-methyl-N-vanillyl-6-none amid) molecules by cleaving the alkyl group linked to the amide (10).After HPP treatment, samples exhibited no significant changes ($P < 0.05$) in CAP but a substantial rise in DHC ($P < 0.05$), peaking at 600 MPa with CAP of 42.68 ± 1.11 mg/100 g and DHC of 10.71 ± 0.01 mg/100 g. Similarly other finding also reported minimal CAP and DHC alteration after HPP (6). HPP may cause incremental temperature increases up to 3°C every 100 MPa (46). Pressurization may enhance extractability and stabilize the components under pressure, including oxidation inhibitors to prevent AA oxidation (47).

The scoville Heat Unit (SHU) measures chilli pungency. A considerably high Scoville heat unit (SHU) was observed in Table 5, Non-pungent (0 SHU), mildly pungent (150−3,000 SHU), moderately pungent (3,000–25,000 SHU), highly pungent (25,000–70,000 SHU), very highly pungent (80,000–1,000,000), and super pungent (>1,000,000 SHU) describe chilli pepper pungency level (48). The SHU ranged from 545886 ± 41708 to 723739 ± 15849, with the thermally treated samples being the lowest and HPP at 600 MPa as the highest.

3.6. Ascorbic Acid, Total Phenolic Content, and DPPH Radical Scavenging Activity (RSA)

Ascorbic Acid (AA), Cumulative Phenolic Content (CPC), and DPPH Radical Scavenging Activity (RSA) are important bioactive characteristics. As shown in Table 6, the bioactive characteristics of control, HPP, and thermal treatment varie.

Table 6 . Ascorbic Acid (AA), Cumulative Phenolic Content (CPC), and DPPH Radical Scavenging Activity (RSA).

Parameter measured	Untreated	High pressure (6 min)			Thermal (80°C 10 min)
		200 Mpa	400 Mpa	600 Mpa	
Ascorbic Acid (mg/g)	401.623 ± 2.89^{d}	431.126 ± 1.55^{c}	470.12 ± 1.93^{a}	445.20 ± 0.29^{b}	321.93 ± 0.51^{e}
(CPC) Cumulative phenolics acid (mg GAE/100 g)	118.69 ± 3.40^{d}	153.48 ± 2.50^{b}	181.71 ± 0.11^{a}	169.56 ± 0.67^{a}	137.20 ± 0.68^{c}
RSA (%)	52.70 ± 0.066^{b}	74.66 ± 0.003^{a}	76.91 ± 0.002^{a}	68.92 ± 0.001^{a}	46.62 ± 0.186^{b}

$^{a, b, c, d, e}$ indicates significantly different values ($p < 0.05$) between column

Thermal treatment reduced ascorbic acid, while pressure treatment increased it, with a threshold of 400 MPa; at 600 MPa, levels decreased but remained higher than those of the control (Table 6). Significant differences ($P < 0.05$) were observed for all AA samples. HPP increased the ascorbic acid content from up to 16.68%, while thermal pasteurization reduced it to 25.15 %. A parallel study observed that pressure increased ascorbic acid content in fresh peppers compared with untreated samples (47) A similar trend was observed in parallel studies: Pressure significantly increases ascorbic acid content in fresh peppers compared with untreated samples (47). TT decreased strawberry puree AA content where as HPP significantly increased it (49); thermal pasteurization reduced tomato puree AA content from 50 – 90% (50,51).

Significant differences ($P < 0.05$) in Cumulative Phenolic Content (CPC) were observed among most samples, except at 400 and 600 MPa, where the CPC peak was observed (Table 6). This trend is consistent with findings of other studies: HPP increased CPC by 6.7% in red pepper paste (52), up to 9.8% in bell peppers (53), and in HPP-treated pineapple puree at 200-600 MPa and 50°C, with reductions at higher temperatures (54). Pressure may facilitate the liberation of matrix-bound phenolics through pressure drop and hydrophobic interactions, thereby increasing CPC (52,55). Interestingly, the thermal results significantly ($P < 0.05$) increased the CPC (Table 6). Both supporting and contrasting findings suggest the potential for heat to either increased or decrease the CPC. (56). Supporting evidence suggest enzyme inhibition, matrix dehydration, and inactivation of polyphenol oxidase may contribute to CPC increase (15,43,51). The sample form (dried or paste), pungency (pungent or sweet), and heating method also affect CPC. Heating dried chilli powder increases CPC, but chilli paste decreases it (15), Heating pungent peppers increase CPC value (7.4–137%) but reduces it in non-pungent ones (1.6–26.9%) (15); boiling, steaming, roasting, and stir-frying reduce CPC to varying degrees (45). These variations highlight the need for further research to better understand the synergistic influence of parameters beyond processing treatments.

The RSA was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH). As shown in Table 6, HPP-treated samples contrasted thermal and control results with a significant difference ($P < 0.05$) in RSA inhibition (68.92–76.91%). Heating methods negatively affect RSA differently: steaming (23.5–30.3%), roasting (11.6–15.4%), and stir-frying (4.6–15.8%) (57). RSA values vary depending on the cultivars, seasons, pre- and post-harvest conditions, chemical

components, ripeness, and pungency (57,58). Spicy peppers have RSA values of 76-90%, where as sweet peppers have 44-88% (20).

4. Conclusions

Both high-pressure processing (HPP) and thermal treatment (TT) have a exhibited substantial influence on various properties, with HPP producing superior results. The pH and Aw of the samples remained consistent regardless of the treatment. TT negatively affected colour quality, while HPP enhances it. Texture analysis revealed distinct treatment effects across various parameters. Although a 5-log reduction was not achieved, the microbial inactivation effect was most pronounced in thermally treated, followed by HPP treatments. Although all samples were within the range of highly pungent peppers, Capsaicin (CAP) and dihydrocapsaicin (DHC) concentrations of TT fell significantly. HPP demonstrated a considerable increase in ascorbic acid (AA) and cumulative phenolic content (CPC), which impact radical-scavenging activity (RSA). The AA, TPC, and RSA values of HPP at 400 MPa yield the best results, where 600 MPa had the most potent pungency properties based on CAP, DHC, and SHU. In summary, HPP treatment at 400 - 600 MPa can preserve quality of chilli pastes better compared to TT (80℃, 10 mins) without safety compromises. Future research should explore the effects of reducing the pH and incorporating natural ingredients as additives in HPP-processed chili pastes.

Acknowledgements

This research was supported by the Universiti Putra Malaysia undera grant with Project No. GP/2020/9693500.

Author Contributions

Each author must have contributed significantly to the idea or design of the work, the collection, analysis, or interpretation of data, the writing or substantial revision of the work, and approval of the submitted version; M.A.N.K. idea or design of the work, writing; A.B.P. collection, analysis, or interpretation of data, writing; P.K.C. Collection, analysis, or interpretation of data, writing; A.M. Writing; E.M.A. Substantial revision of the work; N.M.A. idea or design of the work, substantial revision of the work

Funding

This work was financially supported by the Universiti Putra Malaysia under the grant with Project No. GP/2020/9693500.

Institutional Review Board Statement

Not applicable

Data Availability Statement

None

Conflicts of Interest

No conflict of interest to declare.

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