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FULL-LENGTH RESEARCH ARTICLE

High Pressure Processing Induced Changes in Bioactive Compounds, Antioxidant Activity, Microbial Safety and Color Attributes of Coriander Paste

Prerna Nath[1] · S. J. Kale[1] · O. P. Chauhan[2] · R. K. Gupta[3]

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Abstract An attempt was made to quantify the effect of high pressure processing (HPP) (at 50, 200, 400 and 600 MPa) and thermal processing (90 °C) on bioactive compounds and microbial safety of coriander paste. Total soluble solids (TSS) and pH of untreated coriander paste was 7.8 and 6.21°brix, respectively. No significant ($\alpha = 0.05$) difference was observed in pH or TSS after processing and during storage. Phenolic content increased by 0.99 and 1.10 % when treated at 400 and 600 MPa, respectively. Flavonoid content in high pressure (HP) processed ($\geq$400 MPa) sample was significantly ($\alpha = 0.05$) higher than thermally processed sample. The untreated coriander paste had ascorbic acid content of 1.32 mg/g which decreased to 1.17 and 0.50 mg/g on HP (600 MPa) and thermal processing, respectively. Chlorophyll *a* decreased from 1.68 mg/g to 1.58 and 1.21 mg/g in HP (600 MPa) and thermally processed sample, respectively. Complete enzyme [Polyphenol Oxidase (PPO) and Peroxidase (POD)] inactivation was observed after thermal processing, whereas pressure $\geq$400 MPa required to reduce enzyme activity significantly ($\alpha = 0.05$). Lightness L^* value decreased significantly ($\alpha = 0.05$) with pressure. Total plate count reduced from 3.0×10^3 CFU/g to zero and 1×10^1 CFU/g in thermally and HP processed (600 MPa) samples, respectively. Storage study (at 4 °C for 45 days) showed that bioactive compounds and antioxidant activity in both thermally and HP processed samples decreased during storage, but this decrease was more severe in thermally processed samples. Thus, study reveals that HPP is more effective at $\geq$400 MPa, in terms of retention of bioactive compounds, than the thermal processing.

Keywords High pressure processing · Thermal processing · Coriander · Enzyme · Chlorophyll

Abbreviations
b^*: Blueness or yellowness; C^*: Chroma; °C: Degree Celsius; GAE: Gallic acid equivalent; g: Gram; HPP: High pressure processing; HP: High pressure; h^*: Hue angle; L^*: Lightness; MPa: Megapascal; mg: Milligram; ml: Millilitre; min: Minute; %: Percent; POD: Peroxidase; PPO: Polyphenol oxidase; a^*: Redness or greenness; TFC: Total flavonoid content; TPC: Total phenolic content; TSS: Total soluble solids; WI: Whitening Index;

Introduction

Past few decades have observed changing trends in food habits, and thus, food processing, preservation, product development and quality assurance which consequently have given rise to consistent research on possible alternatives for available food preservation techniques. Traditionally, foods have been preserved by thermal treatments that pose the advantages of preventing spoilage and potential human diseases by inactivating microorganisms

✉ Prerna Nath
prernanath3185@gmail.com

[1] HCP Division, ICAR-Central Institute of Post-Harvest Engineering and Technology, Abohar, Punjab 152116, India

[2] Fruits and Vegetables Technology Division, Defence Food Research Laboratory, Mysore 570011, India

[3] ICAR-Central Institute of Post-Harvest Engineering and Technology, Ludhiana, Punjab 141004, India

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and enzymes of interest such as pectinmethylesterase for maintaining cloud formation in fruit juices and thus help in increasing the shelf life. But, on the other hand, it also possess some limitations such as impaired organoleptic quality in orange juice [6], loss of color and undesirable physical and chemical changes [2] and loss of heat liable nutrients such as ascorbic acid [9].

In view of this non-thermal food preservation, technologies such as high pressure processing have come up in a big way in recent times for food processing. This technology as the name suggest processes food products much below the temperatures involved in conventional processing; thus, food flavors, vitamins, minerals and other organoleptic properties undergo minimal or no changes [3, 8, 24].

The sole principle behind the use of this technology is to inactivate undesirable microorganisms present in the food and also certain enzymes of interest without affecting the nutritional and sensory components that are normally affected during severe heat treatment. Non-thermal processing of food products is therefore being developed as an alternative to traditional thermal food processing procedures [16].

HPP technology offers the food processing industry with great opportunity to undergo intense research in new product development with higher nutritional profile, good taste and texture, perfect aromas and ultimately more shelf life. HPP uses water as a medium to transmit pressures from 300 to 700 MPa to foods resulting in a reduction in microbial loads and thus extending shelf life [19]. Pressure is transmitted uniformly (in all directions simultaneously), thereby helping the food to retain its original shape and texture even at higher pressures.

HPP leads to minimal changes in nutritional value of foods along with it helps in maintaining the 'fresh'-like characteristics of foods by eliminating degradation caused by use of high temperatures. In comparison with thermal processing, HPP results in foods with fresher taste, better esthetic value, texture and nutrition. HPP can be conducted at ambient or refrigerated temperatures, thereby eliminating thermally induced cooked off-flavors and off-taste. This technology can prove to be a boon for heat-sensitive food products.

HPP has been extensively studied and explored by many food scientists and nutritionists all over the world in paste and purees: peach puree [4], pumpkin puree [10], nectarine puree [11], apple puree [15], strawberry puree and blackberry puree [19], tomato and carrot purees [20]. Paste and purees belong to a class of convenience foods for seasonal and perishable nature of fruits or vegetables. Purees and pastes can be used in variety of cuisines for different product developments such as jams, jellies, ketchups, chutneys, gravy thickener, appetizers, dips and conserves. Commercialization of pressure-treated pastes and purees would enable the consumers to avail the benefits of seasonal fruits and vegetables. Moreover, it provides shelf-life extension to the product.

However, there is no literature available on processing and preservation of coriander in paste form using non-thermal HPP technology. Therefore, in light of the above considerations and facts regarding HPP, coriander was explored with the objective of increasing its shelf life and for its easy availability during processing and also for developing microbiologically safe and quality coriander paste with improved nutritional profile in comparison with thermally processed paste or puree.

Materials and Methods

Raw Material and Preparation of Paste

Fresh coriander leaves were procured from a local market of Mysore in the state of Karnataka, India. Coriander leaves were separated from stems, washed in running tap water and ground using a mixer grinder (Preethi Heavy Duty Mixer Grinder Model, MG142) to yield uniform paste. The paste obtained was packed in low-density polyethylene pouches (75 μ thickness and 50 g pack size) and processed (HPP and thermal) immediately.

High Pressure Processing

A laboratory scale high pressure food processing system (ISO-LAB FPG9400, Stansted Fluid Power Ltd., Stansted, UK) consisting of a high pressure vessel (2 l capacity) with dual high pressure pumps and pressure intensifiers which work simultaneously was used to achieve and maintain the desired pressure in the pressure vessel. The system had a maximum operating pressure of 1000 MPa with provisions for temperature and time variation. The high pressure vessel was surrounded by a liquid circulating jacket connected to a heating–cooling system. The pressure transmitting fluid used was 30 % mono-propylene glycol (supplied by M/S Hydraulic on Systems, Ahmedabad, India). The ramp rates for pressurization and decompression were set at 600 and 1000 MPa/min, respectively. The initial temperature increase during pressure buildup (about 2–3 °C/100 MPa) was taken into consideration in order to achieve the desired operating temperature during pressurization. Pressure and temperature were constantly monitored and recorded (at 1-s intervals) during the process using a SCADA (supervisory control and data acquisition)-based software (Stansted Fluid Power Ltd., Stansted, UK).

The coriander pastes samples (50 g) were processed at four different pressure levels of 50, 200, 400 and 600 MPa for a period of 5 min at 20 °C. The paste was analyzed immediately after HPP for its effect on physicochemical properties.

Thermal Processing

Flexible polyethylene bags filled with the sample (50 g) were immersed in a water bath (Medica Instrument Mfg Co.) for isothermal in-bag pasteurization at 90 °C ± 2 for a residence time of 5 min. The coriander paste sample without any treatment was considered as a control sample.

Storage Study

After processing treatments, the flexible polyethylene bags containing coriander paste (control, HP processed and thermally processed samples) were stored at refrigerated temperature (4 °C) for 45 days. The samples were analyzed at an interval of 15 days to assess the effect of the type of processing on coriander paste stability during storage.

PhysicoChemical Analysis

Total soluble solids (°brix) of coriander paste were measured with handheld refractometer (Atago, Japan). The pH of the samples was measured using a pH meter (Century, Model CP931, Bangalore, India). Ascorbic acid was determined as described by Ranganna [21].

Extraction of Phenolics, Flavonoids and Antioxidants

For extraction of total phenolics and antioxidants, 5 g of homogenized sample was extracted twice with 30 ml of 80 % ethanol, by sonicating for 45 min in the dark. Five grams of homogenized sample was sonicated for 45 min in the dark with 30 ml of 80 % ethanol; the extraction was carried out twice till the residue was colorless. The extract was centrifuged for 15 min at 10,000×*g* at 4 °C followed by vacuum concentration in rota-evaporator at 40 °C and stored at 20 °C till further analysis. This concentrated sample was then used as sample extract for estimation of bioactives such as total phenolics, flavonoids and antioxidant activity. This was followed by centrifugation for 15 min at 10,000×*g* at 4 °C. The supernatant was then vacuum concentrated at 40 °C in a rota-evaporator and stored at −20 °C till further analysis. The concentrated sample was used as sample extract for estimation of total phenolics and hydrophilic antioxidant activity.

Total Phenolic and Flavonoid Content

Total phenolics were estimated spectrophotometrically using Folin–Ciocalteu reagent [24]. Results were expressed as gallic acid equivalent (mg GAE/100 ml). Flavonoid content was measured using aluminum chloride method [27]. The absorbance of the solution was measured using UV–Vis spectrophotometer (UV-1601, SHIMADZU). The results were expressed as quercetin equivalent (mg QE/100 ml).

Total Antioxidant Activity

Ferric Reducing Antioxidant Power (FRAP)

FRAP assay was performed according to the procedure described by Benzie and Strain [5]. The FRAP stock solution included 300 mM acetate buffer, pH 3.6, 10 mmol l^{-1} 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution in 40 mmol l^{-1} HCl and 20 mmol l^{-1} $FeCl_3.6H_2O$ solution. The fresh working solution was prepared upon mixing them in a ratio 10:1:1 (v/v/v), respectively, and incubated at 37 °C in a water bath before using; 3 ml of the FRAP solution was mixed with 100 µl of sample extract in a test tube for 10 min in dark condition. Reduction in the ferric tripyridyltriazine to the ferrous complex formed an intense blue color which was measured in a UV–Vis spectrophotometer (UV-1601, SHIMADZU) at 593 nm at the end of 4 min. The results were corrected for dilution and expressed as µmol TE/100 ml.

DPPH Assay

DPPH (2, 2-diphenyl picrylhydrazyl) assay was carried out on the basis of scavenging ability of antioxidants toward the stable radical DPPH [7]. A 3.9 ml aliquot of a 0.0634 mmol l^{-1} of DPPH solution, in methanol (95 %), was added to 100 µl aliquot of sample extract and shaken vigorously. Change in the absorbance of the sample extract was measured at 515 nm for 30 min in a UV–Vis spectrophotometer (UV-1601, SHIMADZU). The antioxidant activity is expressed as percentage inhibition of the free radical calculated using the equation:

$$\text{Inhibition}\,(\%) = \frac{(\text{Abs}_{t=0\text{min}} - \text{Abs}_{t=30\text{min}})}{\text{Abs}_{t=0\text{min}}} \times 100$$

where $\text{Abs}_{t=0\ \text{min}}$ was the absorbance of DPPH radical solution at zero minutes and $\text{Abs}_{t=30\ \text{min}}$ was the absorbance of sample after 30 min. Values were calibrated against standard trolox (100–1000 µmol l^{-1}). Methanol (9) served as a blank. The results were expressed as µmol TE/100 ml.

Extraction and Estimation of Chlorophyll

Chlorophyll content in coriander paste was measured as per the method described by Ranganna [21]; 10 g sample was extracted twice with 50 ml of acetone by stirring and sonicating for 20 min in the dark till the residue was colorless. The filtered contents were extracted with 50 ml of diethyl ether followed by the addition of water to 5–10 times to remove the acetone layer. The ether extract was transferred to a 100-ml volumetric flask and made up the volume with ether, and Na_2SO_4 was added to remove the traces of moisture. The absorbance of the solution versus blank at 660 and 642.5 nm was measured immediately using UV–Vis spectrophotometer (UV-1601, SHIMADZU). The total chlorophyll content, chlorophyll *a* and chlorophyll *b*, was calculated using following formulae:

$$\text{Total chlorophyll}, \left(\frac{\text{mg}}{\text{litre}}\right) = (7.12 \times \text{OD at } 660\,\text{nm}) + (16.8 \times \text{OD at } 642.5\,\text{nm}) \quad (1)$$

$$\text{Chlorophyll } a, \left(\frac{\text{mg}}{\text{litre}}\right) = (9.93 \times \text{OD at } 660\,\text{nm}) - (0.777 \times \text{OD at } 642.5\,\text{nm}) \quad (2)$$

$$\text{Chlorophyll } b, \left(\frac{\text{mg}}{\text{litre}}\right) = (17.6 \times \text{OD at } 642.5\,\text{nm}) - (2.81 \times \text{OD at } 660\,\text{nm}) \quad (3)$$

Extraction and Estimation of Enzymatic Activity

Polyphenol Oxidase (PPO)

PPO activity in coriander paste was measured as per the method described by Gonzalez et al. [12]. For PPO activity, 2 g of coriander paste sample was homogenized at 0 °C in 20 ml of 0.1 M sodium phosphate buffer (pH 7). Extract was filtered through muslin cloth and centrifuged at 0 °C for 20 min at 0 °C for 20 min at 1310×*g*. The supernatant was suitably diluted with a known volume of 0.02 M sodium phosphate buffer (pH 2). Four milliliters of 0.1 M phosphate buffer and 0.8 ml of catechol (0.055 g dissolved in 100 ml distilled water) was dispensed in four test tubes. One milliliter of distilled water was added in one test tube as sample blank, while in others 1 ml of sample extract was added. The absorbance was measured after 1, 2 and 3 min at 410 nm using UV–Vis spectrophotometer (UV-1601, SHIMADZU).

Peroxidase (POD)

POD activity in coriander paste was measured as per the method described by Gonzalez et al. (2000) [12] (POD). The samples (2 g) were homogenized at 0 °C with 20 ml of 0.1 M potassium phosphate buffer (pH 6.5). Extract was filtered through muslin cloth and centrifuged at 0 °C for 20 min at 2166×*g*. The resultant filtered extract containing the enzyme (0.12 ml) was added with 3.48 ml substrate solution [0.1 ml guaiacol (99.5 %) + 0.1 ml hydrogen peroxide (30 %)] and mixed in a vortex. POD activity was measured from the rate of change of absorbance at 470 nm after 3 min.

Instrumental Color

Surface color of the coriander paste samples was recorded using a color meter (Mini Scan XE Plus, Model 45/0-S, Hunter Associates Laboratory, Inc., Reston, VA) as reflected in CIELAB (L^*, a^*, b^*) color space. All the measurements were referenced to the CIE (Commission Internationale de 1'Eclairage) using the standard illuminant D65 and 10° observer, and the equipment was calibrated using white and black standard ceramic tiles. In addition, hue angle (Eq. 1), chroma (Eq. 2) and whitening index (WI) (Eq. 3) were calculated using following equations.

$$h* = \tan^{-1}\left(\frac{b*}{a*}\right) \quad (4)$$

$$C* = \sqrt{a*^2 + b*^2} \quad (5)$$

$$\text{WI} = \sqrt{(100 - L*^2) + a*^2 + b*^2} \quad (6)$$

Microbiological Evaluation

Microbiological analysis of the samples was carried out for standard plate counts, yeast and mold, total coliform, *Salmonella* and *Shigella* according to APHA [1] procedures. In order to isolate the microorganisms, various bacteriological media chemicals and reagents used in the present study were obtained from Hi-media. All media used in the present study were prepared according to the instructions provided by the manufacturing firms and checked for sterility.

Characterization of Salmonella and Shigella Species by Conventional Method

The strains analyzed were *Salmonella enterica typhimurium* MTCC 88 (India) and *Shigella flexneri* ATCC 5160 (USA). A loop full of 24-h-old culture from nutrient agar slant was streaked on Deoxycholate Citrate Agar (DCA), Salmonella–Shigella Agar (SS agar) and xylose lysine deoxycholate agar (XLD) plates and was incubated at 37 °C for 24 h. Presumptive colonies were further purified and subjected to biochemical tests.

Statistical Analysis

Duncans multiple range test (DMRT) was performed to evaluate the statistical differences in the quality attributes of coriander paste as affected by HP and thermal processing. Treatments were compared at 5 % level of significance ($\alpha = 0.05$). SPSS statistical software version 16.0 (SPSS, INC., Chicago, USA) was used to conduct the test.

Results and Discussion

pH and Total Soluble Solids (TSS)

Freshly prepared coriander paste had pH and TSS as 6.21 and 7.8°brix, respectively. Results revealed that no apparent difference was observed in pH or TSS immediately after HP processing and during storage. Similar results were obtained by [11] in nectarine puree treated at 450 and 600 MPa. However, an increase in pH and TSS was observed by Sanchez-Moreno et al. [23] in tomato puree at 400 MPa at 25 °C for 15 min.

Total Phenolic Content (TPC)

Phenolic compounds are important contributors to functional quality and have most important role in counteracting reactive oxygen species (ROS), thus minimizing molecular damage. The effect of processing on total phenolic content (TPC) is presented in Table 1. Both HP and thermal processing significantly ($\alpha = 0.05$) affected the phenolic content. Thermal treatment resulted in significant ($\alpha = 0.05$) decrease (by 47 %) in the levels of phenolic compounds. Processing at low pressure of 50 MPa caused a slight decline (by 0.20 %), whereas higher pressure treatments ranging from 400 to 600 MPa caused a significant increase of 0.99 and 1.10 %, respectively. Increased levels of phenolic content with increasing pressures can be explained by the fact that HP processing caused disintegration of the cellular matrix. These results are in good agreement with the previous reports where HP processing caused an increase in TPC by around 10 % in tomato puree (600 MPa), blackberry (600 MPa) and nectarine puree (450 and 600 MPa) [9, 18, 19]. Onion pieces had a 12 % increase in total phenolics when processed at 100 and 400 MPa combined with high (50 °C) and low (5 °C) temperatures [22]. Thus, it is evident from the results that phenolic compounds were either unaffected or actually increased in concentration and/or extractability following treatment with high pressure. During storage, a significant decrease was observed in both HP and thermally processed samples (Fig. 1).

Table 1 Effect of high pressure processing and thermal treatment on the quality attributes of coriander paste

Treatment	Bioactive compounds						Antioxidant activity		Enzyme activity	
	Chl *a* (mg/g)	Chl *b* (mg/g)	TC (mg/g)	TPC (mg GAE/g)	TFC (mg catechin/g)	AA (mg/g)	DPPH (%)	FRAP (μmol TE/g)	PPO (Abs units/min)	POD (Abs units/min)
Control	1.68 ± 0.003^{c}	0.69 ± 0.006^{f}	2.50 ± 0.01^{e}	9.01 ± 0.01^{b}	5.02 ± 0.02^{c}	1.32 ± 0.04^{f}	37.68 ± 0.04^{c}	25.63 ± 0.01^{c}	260.56 ± 0.1^{c}	165.21 ± 0.2^{c}
50 MPa	1.66 ± 0.003^{d}	0.68 ± 0.005^{e}	2.47 ± 0.01^{d}	8.99 ± 0.005^{b}	5.00 ± 0.03^{b}	1.24 ± 0.01^{e}	36.96 ± 0.02^{b}	24.82 ± 0.02^{b}	265.48 ± 0.1^{d}	168.23 ± 0.2^{d}
200 MPa	1.65 ± 0.006^{d}	0.67 ± 0.002^{d}	2.46 ± 0.00^{d}	9.03 ± 0.01^{c}	5.03 ± 0.03^{c}	1.22 ± 0.03^{d}	36.88 ± 0.04^{b}	24.74 ± 0.04^{b}	274.77 ± 0.05^{e}	169.41 ± 0.2^{e}
400 MPa	1.60 ± 0.007^{c}	0.65 ± 0.004^{c}	2.43 ± 0.007^{c}	9.10 ± 0.007^{d}	5.11 ± 0.01^{d}	1.20 ± 0.01^{c}	37.83 ± 0.03^{c}	25.71 ± 0.01^{c}	165.58 ± 0.04^{b}	130.21 ± 0.2^{b}
600 MPa	1.58 ± 0.004^{b}	0.63 ± 0.005^{b}	2.40 ± 0.008^{b}	9.11 ± 0.02^{d}	5.17 ± 0.01^{e}	1.17 ± 0.01^{b}	38.14 ± 0.04^{d}	26.01 ± 0.01^{d}	98.75 ± 0.06^{a}	86.48 ± 0.1^{a}
Thermal	1.21 ± 0.004^{a}	0.31 ± 0.003^{a}	1.61 ± 0.006^{a}	5.15 ± 0.01^{a}	2.16 ± 0.01^{a}	0.05 ± 0.004^{a}	20.42 ± 0.02^{a}	15.17 ± 0.01^{a}	ND	ND

Values (mean ± SD) followed by the same letter in the same column are not significantly different at 5 % level of significance

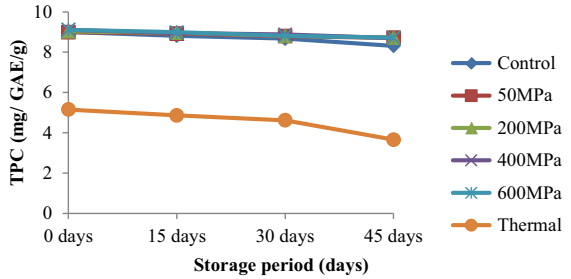

Fig. 1 Effect of HP and thermal processing on total phenolic content of coriander paste during storage period (45 days/4 °C)

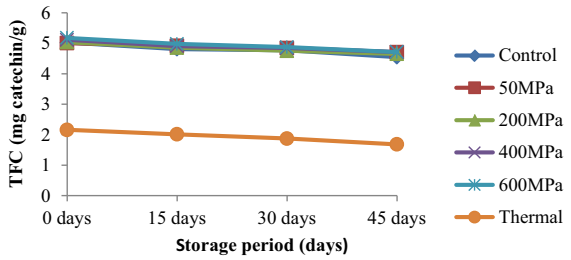

Fig. 2 Effect of HP and thermal processing on the total flavonoid content of coriander paste during storage period (45 days/4 °C)

Total Flavonoid Content (TFC)

Flavonoids are a group of secondary metabolites that are known to act as potent antioxidants and thus have a protective role to play against many degenerative human diseases such as cardiovascular diseases, type II diabetes and cancers. The effect of processing on TFC is presented in Table 1. The TFC in HP processed coriander pastes increased by 3 % at higher pressure range (400–600 MPa) whereas reduced significantly ($\alpha = 0.05$) in thermally processed sample by 56.98 %. Among the four pressures evaluated, 600 MPa showed highest recovery of flavonoids. Increase in TFC with pressure from 400 to 600 MPa might be due to their higher extractability on application of pressure treatments. However, a significant decrease was observed during storage period of coriander paste (Fig. 2). Roldan-Marin et al. [22] reported an increase of 26 % in TFC in onions treated at pressure levels of 100 and 400 MPa.

Ascorbic Acid

Ascorbic acid is considered as a quality indicator in fruits and vegetable products as it is a sensitive bioactive compound that gives an indication about the status of other vitamins of importance. Occurrence of high ascorbic acid content is beneficial, as the presence of natural ascorbic acid acts as an antioxidant and thus helps circumvent the problem associated with oxidation of other bioactive compounds, thereby improving their stability. The effect of processing on ascorbic acid is presented in Table 1. The untreated coriander paste had ascorbic acid content of 1.32 mg/g which decreased significantly to 1.17 and 0.50 mg/g on HP processing (600 MPa) and thermal processing, respectively. However, the degradation was higher with thermal processing showing a significant decrease ($\alpha = 0.05$) of 61 %, whereas HPP resulted in a loss of 6–12 % with increase in pressure levels from 50 to 600 MPa. During storage at low temperature, ascorbic acid decreased significantly ($\alpha = 0.05$) over a storage period of 45 days at 4 °C (Fig. 3). In a study focusing on tomato puree, Patras et al. [20] determined that there were significant reductions in vitamin C in all processed products, whether HPP or thermal. However, thermally processed samples retained only 54 % of the original vitamin C content, while HPP processing at 600 MPa resulted in 94 % of the original content. The same authors also determined that vitamin C was not detectable in any processed carrot puree samples.

Chlorophyll Content

Chlorophyll is the green molecule in plant cells that carries out the bulk of energy fixation in the process of photosynthesis. Chlorophyll itself is actually not a single molecule but a family of related molecules, designated as

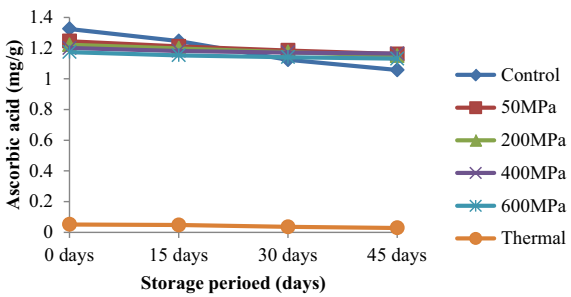

Fig. 3 Effect of HP and thermal processing on ascorbic acid of coriander paste during storage period (45 days/4 °C)

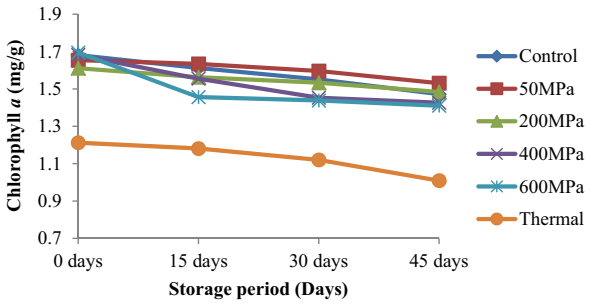

Fig. 4 Effect of HP and thermal processing on the chlorophyll *a* of coriander paste during storage period (45 days/4 °C)

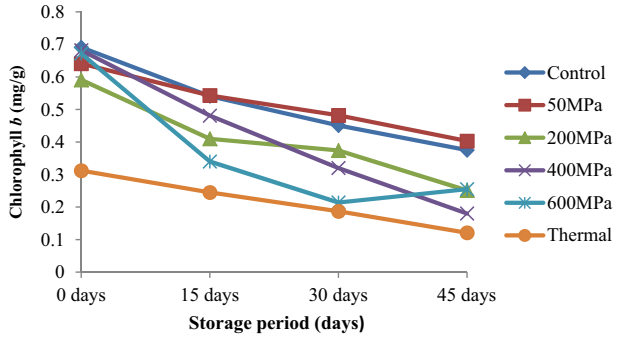

Fig. 5 Effect of HP and thermal processing on chlorophyll *b* of coriander paste during storage period (45 days/4 °C)

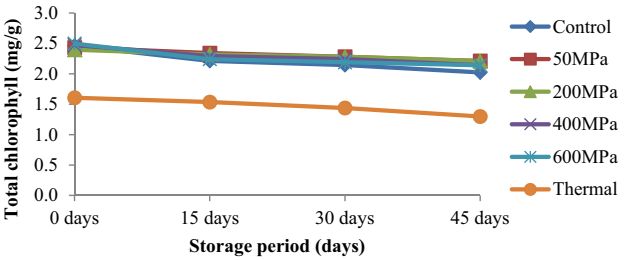

Fig. 6 Effect of HP and thermal processing on the total chlorophyll of coriander paste during storage period (45 days/4 °C)

chlorophyll *a*, *b*, *c* and *d*. Chlorophyll *a* is the molecule found in all plant cells, and therefore, its concentration is what is reported during chlorophyll analysis. The effect of processing on chlorophyll content is presented in Table 1.

Chlorophyll *a* of untreated coriander paste was 1.68 mg/g, and it decreased significantly ($\alpha = 0.05$) to 1.58 and 1.21 mg/g after HP processing (at 600 MPa) thermal processing, respectively. Percent loss in chlorophyll *a* of HP processed (50–600 MPa) samples ranged from 1.25 to 6 %, whereas loss of 28 and 55 % took place in chlorophyll *a* and chlorophyll *b* in thermally treated samples, respectively. Significant decrease ($\alpha = 0.05$) was observed in chlorophyll content (chl *a*, chl *b*, total chl) of all samples during storage period (Figs. 4, 5, 6). A decrease of 17, 62 and 14 % was observed in chl *a*, chl *b* and total chl content at the end of storage period. In other report by Sanchez-Moreno et al. [23], tomato puree samples treated at 400 MPa/25 °C/15 min had the highest content of all carotenoids—β-carotene, γ-carotene, lycopene and lutein. In contrast, tomato puree processed at HPP 500 and 600 MPa for 12 min caused 21 and 56 % loss of total lycopene, respectively, probably due to isomerization of *trans* to *cis* forms. These authors found that lower-pressure levels (100–400 MPa) had no effect on lycopene content and storage of processed (HPP 100–300 MPa) tomato puree at 24 °C for 16 days resulted in only 8–9 % loss.

Total Antioxidant Activity

The pressure stability study of antioxidants is of paramount importance due to their significant role in preventing degenerative diseases such as cardio vascular diseases and cancers. The evaluation of antioxidant activity in food sample is becoming increasingly important in the field of nutraceutical research as it provides useful information with regard to health promoting functional quality of food material without the analysis of each antioxidant compound. In the present study, authors used two in vitro assays, namely FRAP and DPPH assay. Although the above two methods are based on electron transfer reactions, FRAP assay reflects the metal reducing power, whereas DPPH assay reflects the organic radical scavenging power of dietary antioxidants.

The effect of processing on antioxidant activity is presented in Table 1. Thermally processed coriander paste resulted in greater losses and thus reduced antioxidant activity in comparison with HP processed samples. Antioxidant activity of coriander paste hence also dramatically improved after HP processing. The pressure treatment at 400 and 600 MPa showed an increase of 0.32 and 1.48 %, whereas a reduction of 39 and 46 % in FRAP and DPPH values was observed in thermally treated coriander paste samples. A significant increase ($\alpha = 0.05$) was observed in FRAP values ranging from 25.63 to 26.01 µmol TE/g, respectively, in untreated and coriander paste treated at high pressure of 600 MPa. The results demonstrated that HP processing causes structural changes in tissue matrix, thereby releasing bound phenolics, flavonoids and thus antioxidant compounds from fibrous plant matrix into the extracellular environment, accounting for their increased content in the extract. High antioxidant activity is thus a consequence of higher release of phytochemicals from cellular matrix which are ultimately the major contributors. Total antioxidant activity showed a significant decrease ($\alpha = 0.05$) during storage period (Figs. 7, 8).

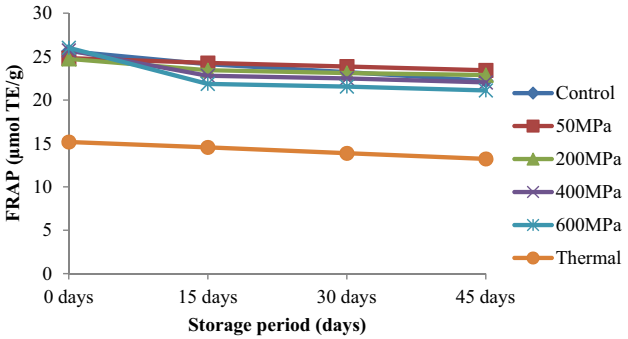

Fig. 7 Effect of HP and thermal processing on the FRAP values of coriander paste during storage period (45 days/4 °C)

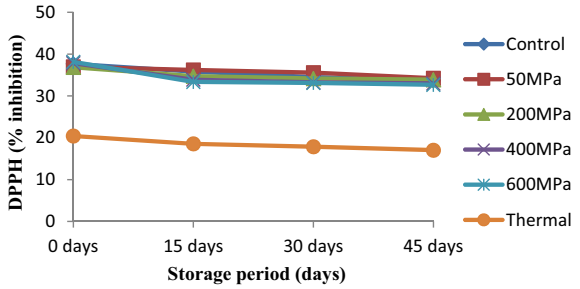

Fig. 8 Effect of HP and thermal processing on the DPPH values of coriander paste during storage period (45 days/4 °C)

The results showed that there was a slight increase in the total antioxidant activity in terms of % radical scavenging activity (RSA) at high pressure treatments of 400 and 600 MPa. The RSA activity of control sample was 37.68 %, whereas it was 38.14 % when processed at 600 MPa. These results are in good agreement with some of the previous reports [11, 18, 19] who reported better retention of antioxidant activity in high pressure-treated blackberry purees, carrot puree and nectarine puree.

Polyphenol Oxidase (PPO) and Peroxidase (POD) Activities

PPO and POD are important enzymes to be considered during processing of fruits and vegetables. They are responsible for bringing out several undesirable quality changes such as flavor losses and discoloration. Therefore, their inactivation is of prior importance for obtaining quality processed fruits and vegetable products. It was observed that HP processing (at different pressures) and thermal processing (at 90 °C for 5 min) affected the enzymatic activity significantly ($\alpha = 0.05$).

The effect of processing on enzymatic activity is presented in Table 1. Complete inactivation of enzymes (PPO and POD) was noticed after thermal processing, whereas lower-pressure treatments (50 and 200 MPa) caused a significant ($\alpha = 0.05$) increase in enzyme activity. The enhanced activity at lower pressures might be attributed to

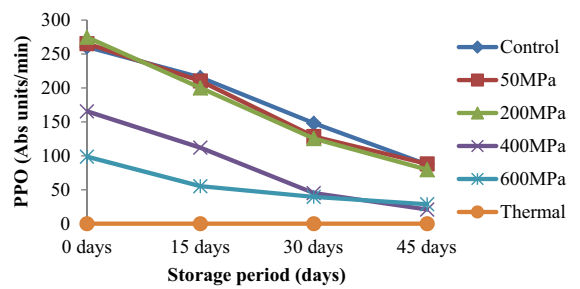

Fig. 9 Effect of HP and thermal processing on PPO of coriander paste during storage period (45 days/4 °C)

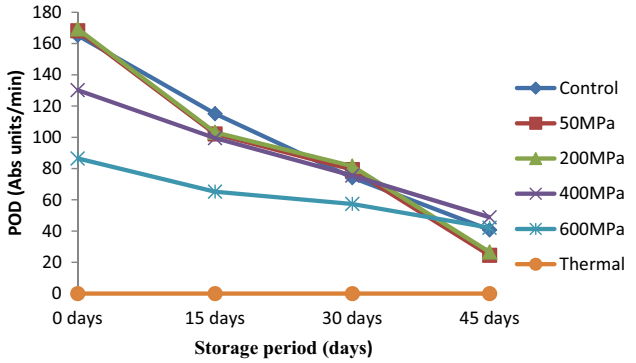

Fig. 10 Effect of HP and thermal processing on POD of coriander paste during storage period (45 days/4 °C)

the pressure-induced membrane damage and the resulting leakage of enzyme and substrate. The mechanism of high pressure inactivation of the enzyme was related to the change of hydrogen bonds between the surrounding water on the surface of protein molecules. Enzymes are proteins in nature with active sites responsible for their entire biological activity, and thus, pressure inactivation of enzymes is possible through disruption of their active sites. Minute changes in active sites of enzymes can result in total loss of its biological activity. Increasing pressures induce disruption of protein structure due to relocation of bonds involved such as hydrophobic and electrostatic; however, HPP does not affect covalent bonds which help enzymes to refold and gain their biological activity after depressurization [13].

On application of higher pressures of 400 and 600 MPa, enzyme activity decreased significantly ($\alpha = 0.05$). A similar trend was observed with POD activity, which increased significantly at lower pressures of 50 and 200 MPa whereas decreased at higher pressures of 400 and 600 MPa. A 21 and 48 % reduction in POD activity was observed at 400 and 600 MPa pressures, respectively. Similarly, a higher decrease of 62 and 79 % was observed in PPO activity when treated at 400 and 600 MPa pressures,

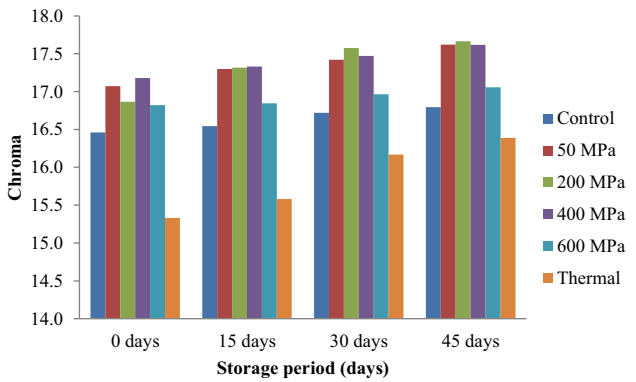

Fig. 11 Effect of HP and thermal processing on chroma of coriander paste during storage period (45 days/4 °C)

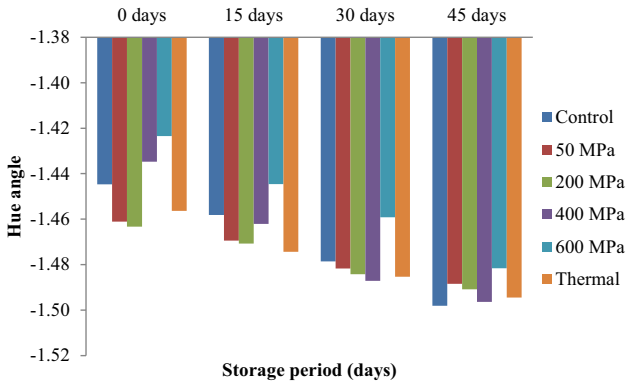

Fig. 12 Effect of HP and thermal processing on hue angle of coriander paste during storage period (45 days/4 °C)

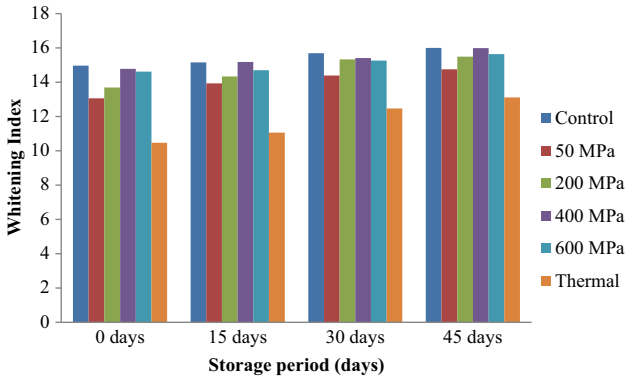

Fig. 13 Effect of HP and thermal processing on whitening index of coriander paste during storage period (45 days/4 °C)

respectively. Weemaes et al. [26] reported a pressure inactivation of PPO in apples treated at 600 MPa and at room temperature (25 °C). Similar observations were made in peach puree [4] and avocado paste [14]. Enzymatic activity decreased significantly ($\alpha = 0.05$) during storage period (Figs. 9, 10).

Color Attributes

Effect of HPP and thermal processing on coriander paste was evaluated by determining the chroma (C^*), hue angle (h^*) and WI of the samples and shown in Figs. 11, 12 and 13. Chroma represents the color intensity of the sample in comparison with gray color with the same lightness, whereas hue angle gives the difference of certain color in comparison with gray color with the same lightness [18]. The WI actually represents the overall whiteness of food products which helps in indicating degree of discoloration caused due to certain food processing operation.

Results (Fig. 11) revealed that C^* value of high pressure processed samples, irrespective of the pressure, was significantly ($\alpha = 0.05$) higher (by 10–12 %) than that of thermally processed sample indicating the decrease in color intensity after thermal processing. It was observed that storage period had no significant effect on chroma of high pressure processed samples, but the C^* value of thermally processed sample increased (by 2–7 %) with storage period. Figure 12 shows that, though not significantly ($\alpha = 0.05$), hue angle values increased with pressure after all storage intervals. No significant difference was observed in hue angle values of pressure processed (at 600 MPa) sample and thermally processed sample. It was also observed that with storage period, hue angle values decreased slightly with the storage period indicating the decrease in lightness as well as fresh-like color of the samples.

Whitening index (WI) (Fig. 13) of high pressure processed samples was found to be significantly higher (by 25–41 %) than that thermally processed sample at any storage interval. It was also observed that, with pressure, WI of the pressure processed samples increased slightly. During storage of all the samples, WI was found to be increased significantly ($\alpha = 0.05$). Increase in WI with storage might be because of residual enzyme activity leading to change in color of coriander paste [18].

Microbiological Quality

One of the objectives of HPP is to inactivate pathogenic and spoilage microorganism such as bacteria, yeasts, molds and viruses, thus producing quality and safe food product [25]. However, the extent of bacterial inactivation also depends on the type of microorganism present, food composition, pH and water activity. The data with regard to microbial count of coriander paste are presented in Table 2. It is evident from Table 2 that high pressure treatments significantly affected the microbial growth. The total plate count for control sample was found as 3.0×10^3 CFU/g, whereas it was nil in the thermally treated sample. A significant decrease ($\alpha = 0.05$) in TPC was observed with increasing pressure levels, whereas it was found to decrease from 3.0×10^3 CFU/g to 1.0×10^1 in coriander paste when treated over a pressure range of 50 to 600 MPa. The total coliform, yeast and mold, Salmonella and Shigella were found to be absent in all the samples irrespective of HP or thermal processing. Several reports exist with regard to pressure inactivation of microorganisms at high pressure showing pasteurization/sterilization effects in different types of products [17]. It is evident from Table 2 that microbial count increased with storage period.

Conclusions

Coriander is used in various culinary preparations, and its infusions have been preferred for various ailments such as diabetes, flatulence, gastric malfunctioning, respiratory

Table 2 Effect of high pressure processing and thermal treatment on the microbial load of coriander paste

	Days	Control	50 MPa	200 MPa	400 MPa	600 MPa	Thermal
Total plate count (CFU/g)	0	3.0×10^3	3.0×10^3	2.5×10^2	2.0×10^1	1.0×10^1	A[a]
	15	4.0×10^3	3.7×10^3	3.0×10^3	3.0×10^2	1.5×10^1	A
	30	5.5×10^4	4.1×10^3	4.5×10^3	3.6×10^3	2.0×10^1	A
	45	6.7×10^4	4.5×10^4	2.6×10^4	4.8×10^3	3.0×10^1	A
Total coliform	A	A	A	A	A	A	A
Yeast and mold	A	A	A	A	A	A	A
Salmonella	A	A	A	A	A	A	A
Shigella	A	A	A	A	A	A	A

[a] A implies absence of microorganism

tract disorders and urinary infections which indicate its importance in human diets. High pressure processing has potential to retain inherent quality characteristics of coriander paste which otherwise get reduced partially or completely during thermal processing. The present study revealed that there was no significant ($\alpha = 0.05$) difference in total soluble solids and pH of the control, high pressure-treated and thermally processed coriander paste samples. These two parameters were almost unchanged during storage also. The total phenolic content and TFC increased significantly ($\alpha = 0.05$), whereas ascorbic acid content, chlorophyll content and antioxidant activity decreased with increasing pressure (400–600 MPa). But the effect of pressure treatments on these parameters was less severe than that of thermal treatment. Study demonstrated that thermal processing brought complete inactivation of enzymes (PPO and POD) unlike high pressure processing. At 50 and 200 MPa pressures, a significant increase in enzyme activity was observed. But further increase in pressure ($\geq$400 MPa) decreased enzyme activity significantly ($\alpha = 0.05$). Both thermal and high pressure processing affected color values significantly ($\alpha = 0.05$). Lightness of the sample decreased, whereas redness and yellowness increased with pressure. Thermal processing brought complete microbial destruction (from 3.0×10^3 CFU/g to nil), whereas high pressure processing reduced it from 3.0×10^3 CFU/g to 1.0×10^1 in coriander paste when treated at 600 MPa. Effect of high pressure processing and thermal processing on coriander paste during storage was also evaluated in the study. Though bioactive compounds and antioxidant activity in both thermally processed and high pressure processed coriander paste samples decreased during storage, this decrease was more severe in case of thermally processed samples. Thus, study underscored the potential of high pressure processing technology (at $\geq$400 MP) for enzyme inactivation and microbial destruction in coriander paste without severe loss in its bioactive and phytochemical compounds.

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