Comparative Evaluation of Hot Water and Steam Blanching Methods on Quality Characteristics of Spinach (*Spinacia oleracea*)

Harshitha VSLP [a++*], Dutta Samit [a#] and P. Srilatha [a†]

[a] College of Food Processing Technology and Bioenergy, Anand Agricultural University, Anand, 388110, Gujarat, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors HVSLP and DS designed, planned and carried out the experiments and performed statistical analysis. Authors HVSLP and PS analysed the data and contributed to the drafting and editing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to standardize hot water and steam blanching for the processing of Spinach, assessing their effectiveness in peroxidase (POD) enzyme inactivation and their impact on key quality attributes including color, pH, total soluble solids (TSS), antioxidant activity, ascorbic acid, oxalate content and microbial quality.

Study Design: A completely randomized design (CRD) was employed, utilizing one-way ANOVA

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[++] *M.Tech Scholar;*
[#] *Associate Professor;*
[†] *Ph. D Scholar;*
Corresponding author: Email: harshihavensla@gmail.com;

and Duncan's multiple range (DMR) tests to evaluate and compare the effect of the blanching method on spinach quality.

Place and Duration of Study: College of Food Processing Technology and Bioenergy, Anand Agricultural University, Anand, between March and Oct 2024.

Methodology: Various hot water blanching parameters (70 °C and 90 °C for 30 s and 60 s) and steam blanching durations (60 s, 120 s, and 180 s) were tested. Peroxidase (POD) activity was analyzed to determine inactivation. Hot water at 90 °C for 30 s and steam for 180 s were identified as optimal. These optimal parameters were then used to assess their impact on various quality attributes.

Results: Statistical analysis revealed that hot water blanching (90 °C, 30 s) and steam blanching (180 s) were equally effective in peroxidase (POD) inactivation, with residual activities of $9.79 \pm 1.05\%$ and $10.50 \pm 0.72\%$, respectively. Greenness (a^* value) improved from -6.51 ± 0.21 (fresh) to -9.86 ± 0.04 (hot water) and -7.15 ± 0.05 (steam). Total oxalate content decreased significantly, from 454.66 ± 45.79 mg/100 g (fresh) to 220.00 ± 44.00 mg/100 g (hot water) and 286.00 ± 22.00 mg/100 g (steam), representing a reduction of nearly 50%. Both blanching methods significantly increased pH and simultaneously reduced total soluble solids (TSS), antioxidant activity, ascorbic acid content, and microbial load.

Conclusion: Blanching significantly enhanced spinach greenness and reduced anti-nutrients (oxalates) and microbial load. This study provides food processors with scientific evidence for standardizing blanching methods ensuring maximum enzyme inactivation with minimal nutrient loss, thus supporting production of minimally processed, nutrient-rich spinach products.

1. INTRODUCTION

Proper nutrition is crucial for maintaining good health, for centuries, green leafy vegetables have been a key dietary component due to their availability, affordability, and low-calorie content. They are resilient to adverse conditions such as droughts (Randhawa et al., 2015). Green leafy vegetables, also known as salad greens, leafy greens, or potherbs, include spinach, amaranth, fenugreek, and drumstick (Joglekar et al., 2014; Kumar et al., 2020). In Indian cuisine, leafy vegetables are used in a variety of dishes, including chutneys, pickles, curries, snacks, and soups (Ray & Ray, 2022).

Spinach (*Spinacia oleracea*), commonly known as '*Palak*', in India, is a major leafy vegetable from the *Amaranthaceae* family. It is cultivated as an annual crop in spring and autumn (Mudau et al., 2019). In 2022-23, global spinach production reached 33 million tonnes, with China as the leading producer (Sharma et al., 2024). Spinach is rich in vitamins, minerals, antioxidants, flavonoids, carotenoids, and phenolic compounds, making it a highly nutritious vegetable (Bhattarai & Shi, 2021).

Spinach is typically processed into canned or frozen forms, where the quality of raw materials and pre-treatments are important (Kamiloglu, 2020). Blanching is a common pre-treatment method, involves exposing vegetables to high temperatures (<100 °C) for a short time to inactivate enzymes and reduce surface microbes. It also helps in maintaining quality of products during long term storage. Usually, blanching was performed by hot water, steam, microwave, or infrared. Among them, hot water blanching and steam blanching are commercially popular due to their simplicity and affordability (Xiao et al., 2017). The efficacy of blanching depends on the time needed to inactivate peroxidase and polyphenol oxidase enzymes (Akyol et al., 2006). Since, peroxidase (POD) is heat-resistant, its inactivation is a key indicator of effective blanching (Xin et al., 2015). Blanching also improves the quality characteristics such as yield and sensory qualities of fruit juices (Adubofuor et al., 2016).

Therefore, blanching is a critical processing step for preserving the quality of spinach, a widely consumed leafy vegetable. While previous studies have examined specific aspects of blanching, such as enzyme inactivation or nutrient retention, they often focus on isolated parameters rather than their combined impact on overall product quality. This study takes a more integrated approach by comparing hot water and steam blanching across multiple quality and safety attributes. By evaluating these factors together, we provide a broader understanding of how blanching methods influence final product

quality. These findings can provide valuable insights to food processors in developing clean-label or minimally processed spinach products with improved consistency and quality.

2. MATERIALS AND METHODS

2.1 Raw Material Preparation

For this study, fresh spinach was procured from a local farmer in Anand, Gujarat, India. The spinach leaves were thoroughly washed under running tap water to remove any dirt and foreign matter. The cleaned spinach leaves were sorted to remove any damaged or wilted leaves. Subsequently, the spinach leaves were cut into smaller pieces (3-4 mm) to facilitate even blanching. Following this, the spinach leaves were divided into two equal batches for the respective blanching treatments and were used for subsequent analysis of quality parameters.

2.2 Blanching

Two blanching methods, hot water and steam blanching, were evaluated using the procedure described by Quaye et al. (2021) with slight modifications. Peroxidase residual activity was measured to determine optimal process parameters. For hot water blanching, fresh leaves were cleaned, cut into small pieces and blanched in hot water at 70 °C and 90 °C for 30 and 60 s. After blanching, the leaves were cooled rapidly in cold water (approximately 5 °C), drained and set aside for further analysis. In steam blanching, cleaned and cut leaves were taken. Then, clean water was filled in a vessel and was left to boil until noticeable steam was observed. When a noticeable amount of steam (100 °C) was produced, the sample was taken in a colander and was placed above the vessel in the steam for 60, 120 and 180 s. The leaves were then cooled quickly in cold water, drained and prepared for analysis. The treatments with the lowest enzyme activity were selected for further study.

2.3 Peroxidase Activity

To determine the adequacy of blanching treatment the residual activity of peroxidase (POD) was measured using the procedure described by Akyol et al. (2006) with slight modifications. Mix 2 g of sample with 8 mL of 0.2 M phosphate sodium buffer (pH 6.5), then centrifuge at 8000×g for 20 min at 4 °C. Collect and store the supernatant at 4 °C. Prepare the substrate solution with 0.5 mL guaiacol, 0.5 mL hydrogen peroxide, and 99 mL of buffer. Combine 0.1 mL of enzyme extract with 3.5 mL of substrate solution, and measure absorbance at 470 nm for 3 minutes using a UV-Visible Spectrophotometer. The specific activity of POD was expressed as the rate of linear change in absorbance over reaction time (A/min). The residual activity of POD was estimated using the following equation:

$$\text{Residual activity } (\%) = \frac{\text{Specific activity of POD in the untreated sample}}{\text{Specific activity of POD in the treated sample}} \times 100$$

2.4 Colour (a* value)

The colour value of spinach was measureds using a Lovibond colorimeter (Model RT 850i, Lovibond) in terms of a* value (Lee et al., 2021).

2.5 pH

The pH was measured using a pH/Mv combination meter (CL 120, Chemi Line) in accordance with the AOAC (2019) standard method.

2.6 Total Soluble Solids (TSS)

TSS of spinach puree was measured using a digital handheld refractometer (OPTi, Bellingham + Stanley) following the AOAC (2019) standard method.

2.7 Antioxidant Activity

The antioxidant activity of spinach was estimated using the DPPH scavenging effect method as described by Ezzat et al. (2020). 100 mg of sample was prepared by adding 1 g of sample in 10 mL of methanol. 100 mg of the aliquot of extract was withdrawn in a test tube and then 2.9 mL of DPPH (0.1 mM) solution was added. After that, the mixture was incubated in the dark for 30 min. Absorbance was measured against the blank at 517 nm in UV-Visible spectrophotometer. DPPH solution was used as blank (control). The antioxidant activity of spinach was calculated by the following formula:

$$\text{Antioxidant activity } (\%) = \frac{\text{Absorbance of blank-Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

2.8 Ascorbic Acid

The ascorbic acid of the samples was estimated using 2, 6-dichlorophenol-indophenol by titration method (Bhat et al*., 2017). In this method, standard ascorbic acid was prepared by weighing 100 mg of L-ascorbic acid was weighed and made up to 100 mL with 3 % HPO_3. Dilute 10 mL of this solution to 100 mL with 3 % HPO_3 (1 mL = 0.1 mg ascorbic acid). For dye solution, 50 mg of sodium salt of 2, 6-dichlorophenol-indophenol was dissolved in 150 mL of hot distilled water containing 42 mg sodium bicarbonate. After cooling, the dye was diluted to 200 mL with distilled water and standardized for estimation at the time of its use. For standardization of dye, take 5 mL of the standard ascorbic acid solution and add 5 mL of HPO_3 and titrate against the dye solution to a pink colour. The dye factor was determined using the following formula:

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}}$$

For sample analysis, prepare a 100 mL solution of 10 g sample in 3% HPO_3, titrate a 10 mL aliquot with the dye and the ascorbic acid content was calculated by using the following formula:

$$\text{Ascorbic acid (mg/10g)} = \frac{\text{Titre value (mL)} \times \text{Dye factor} \times \text{Volume made up (mL)} \times 100}{\text{Aliquot volume (mL)} \times \text{Weight of sample (g)}}$$

2.9 Total Oxalate Content

Total oxalate content was estimated using titration method (Mishra et al., 2017). 1 g of plant material was weighed in electric weighing balance and transferred to 30 mL of 0.5 N H_2SO_4 and was boiled in a water bath for 15 minutes. Then the extract was filtered with Whatman's No. 1 filter paper. Equal volume of deionised water was added. Then 10mL of filtered extract was taken and 40 mL 0.5N H_2SO_4 was added. Final 50 mL of mixture was heated to 60 °C and was titrated against 0.05N $KMnO_4$. The end point was determined by permanent appearance of light pink colour. The total oxalate content was calculated stoichiometrically. The reaction involved was given below:

$$MnO_4^- + 5C_2O_4^{2-} + 8H^+ \rightarrow Mn^{2+} + 10CO_2 + 4H_2O$$
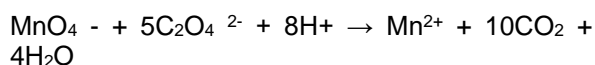
2.10 Aerobic Plate Count

Aerobic plate count was determined using the standard pour-plate method (Adsure et al., 2023). 1 g of sample was pipetted in a sterilized petri-plate (in triplicates) under a sterile environment and then 15 mL of nutrient agar was poured into plates. The plates were gently rotated in a circular motion and the media was allowed to solidify. The solidified plates were inverted and incubated in an incubator at 37 ± 0.5 °C for 48 h and the number of colony forming units (CFU) was recorded. The data was presented as log CFU/g.

2.11 Statistical Analysis

All tests were performed in triplicates, and the results were expressed as mean ± standard deviation. Statistical analysis was done using one-way analysis of variance (ANOVA) and Duncan's multiple range (DMR) test was employed to detect the significance of differences within treatments ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Standardization of Blanching Methods

Blanching treatments were standardized using one-way ANOVA, with results shown in Table 1. Statistical analysis showed that hot water blanching at 90 °C for 30 s and 60 s, and steam blanching for 180 s, were statistically equivalent in peroxidase (POD) inactivation. Although the selected steam blanching time was longer than that required for hot water blanching, this difference was determined through initial experimental observations. In hot water blanching, extending the time beyond 60 s caused leaf browning, which affected visual quality. On the other hand, steam blanching for 60 s and 120 s resulted in high residual POD activity (approx. 44% and 26%), indicating that the enzyme was not fully inactivated at shorter times. Thus, a longer duration of 180 s was needed to match the effectiveness of hot water blanching. These findings are supported by Ijod et al. (2025) and Moscetti et al. (2019), who also reported that steam requires longer exposure to achieve similar enzyme inactivation due to slower heat penetration.

Bamidele et al. (2017) noted that longer hot water blanching time increased nutrient loss, suggesting shorter time are preferable. Therefore, hot water blanching at 90 °C for 30 s and steam blanching for 180 s were chosen as standardized treatments.

Table 1. Effect of blanching method on peroxidase (POD) residual activity

Blanching method	Temperature (°C)	Time (s)	POD residual activity (%)
Hot water blanching	70	30	27.86 ± 0.96[b]
Hot water blanching	70	60	19.62 ± 0.90[d]
Hot water blanching	90	30	9.79 ± 1.05[e]
Hot water blanching	90	60	9.80 ± 0.54[e]
Steam blanching	-	60	44.30 ± 0.38[a]
Steam blanching	-	120	25.88 ± 0.82[c]
Steam blanching	-	180	10.50 ± 0.72[e]

Values are expressed in mean ± standard deviation (n=3). Values followed by different letters in the same column are significantly different by the Duncan's test at 5 % level of significance

3.2 Effect of Blanching Method on Quality Characteristics of Spinach Puree

Blanching affects the colour which plays significant role in product acceptability. The colour (a* value) of fresh, hot water blanched, and steam blanched leaves was -6.51 ± 0.21, -9.86 ± 0.04, and -7.15 ± 0.05, respectively, indicating that blanching increased greenness. The negative a* values indicates the intensity of greenness. These results are in agreements with findings of Severini et al. (2016), in which it was explained that air removal during blanching increased the greenness, altering surface reflectance and colour. Heat treatment during blanching also causes pigment changes, leading to more uniform colour distribution. In this study, hot water blanched samples exhibited greater greenness than steam blanched ones.

The pH of samples ranged from 6.11 to 6.62, indicating spinach is a low-acid food. There was no significant difference between the blanched samples; however, both blanched treatments exhibited higher pH values compared to fresh leaves, indicating that blanching increased pH. This increase was likely due to the loss of organic acids during the blanching process, which was explained by Martínez et al. (2013) and Adetoro et al. (2020).

Total soluble solids (TSS, °B) were 7.60 for fresh, 6.85 for hot water blanched, and 7.10 for steam blanched samples. A significant difference was observed between the blanched and fresh samples, with the blanched samples showing lower TSS values. Among the blanched treatments, hot water blanching resulted in lower TSS than steam blanching, which suggested greater leaching of soluble components into the water during hot water blanching. Similar observations were reported by Siti et al. (2022) and Martínez et al. (2013), who explained that the leaching of nutrients and other water-soluble compounds into the blanching medium led to reduced TSS values in hot water blanched vegetables compared to fresh and steam blanched samples.

Antioxidant activity of fresh leaves was 68.12 %, decreasing to 48.50 % in hot water blanched leaves and 52.45 % in steam blanched leaves. This shows that blanching led to decrease in antioxidant activity. Meena et al. (2016) also reported decreased antioxidant activity in green leafy vegetables blanched in boiling water, primarily due to leaching. There was no significant difference in both the blanching treatments.

Ascorbic acid, sensitive to light, oxygen, and heat, decreases due to thermal degradation or leaching during blanching (Patel et al., 2016). In this study, ascorbic acid decreased from 48.34 mg/100 g to 34.50 mg/100 g, with a greater loss observed in steam blanching compared to hot water blanching. This high loss in steam blanching may be due to longer processing time.

Spinach, with high oxalate content, can reduce calcium availability (Shkembi & Huppertz, 2021). Fresh spinach had 454.66 mg/100 g of total oxalates. Hot water blanching reduced oxalate content to 220 mg/100 g, while steam blanching reduced it to 286 mg/100 g. There was no significant difference between hot water blanching and steam blanching, indicating that both methods are effective in reducing anti-nutrients like oxalates up to 50 %. The decrease in oxalate content in blanched leaves is mainly due to the leaching of water-soluble oxalates which was also observed by Wang et al. (2018).

Table 2. Effect of blanching method on quality characteristics of spinach

Parameter	Fresh	Hot water blanched	Steam blanched
Colour (a* value)	-6.51 ± 0.21[a]	-9.86 ± 0.04[c]	-7.15 ± 0.05[b]
Ph	6.11 ± 0.05[b]	6.57 ± 0.02[a]	6.62 ± 0.01[a]
TSS (° B)	7.60 ± 0.15[a]	6.85 ± 0.10[c]	7.10 ± 0.06[b]
Antioxidant activity (% DPPH Inhibition)	68.12 ± 2.25[a]	48.50 ± 2.51[b]	52.45 ± 1.18[b]
Ascorbic acid (mg/ 100 g)	48.34 ± 6.90[a]	37.60 ± 0.00[b]	34.50 ± 7.96[c]
Total oxalate (mg/100g)	454.66 ± 45.79[a]	220.00 ± 44.00[b]	286.00 ± 22.00[b]
Aerobic plate count (log CFU/g)	2.38 ± 0.02[a]	0.30 ± 0.31[b]	0.24 ± 0.24[b]

Values are expressed in mean ± standard deviation (n=3). Values followed by different letters in the same row are significantly different by the Duncan's test at 5 % level of significance

In both methods of blanching, 2 log reduction in aerobic plate count was observed. Similar results were noted by Njoroge (2015) in which significant microbial reduction (5 log) was observed in green leafy vegetables blanched at 80-98 °C for 0-10 minutes.

4. CONCLUSION

In conclusion, blanching significantly impacted the quality characteristics of spinach, particularly enhancing greenness (a* value) and reducing anti-nutrients such as oxalates. Hot water blanching improved greenness but caused greater losses soluble solids compared to steam blanching. Steam blanching caused greater loss of ascorbic acid but had slightly lower loss in soluble solids. Both blanching methods effectively reduced oxalate content and microbial load, with no significant difference between them. This study provides a basis for food processors in standardizing blanching methods that achieve maximum enzyme inactivation with minimal nutrient loss, making them suitable for producing minimally processed, nutrient-rich products. The choice of method also should depend on factors such as nutrient preservation or anti-nutrient reduction, equipment availability and processing conditions. Adjustments to parameters can be made with these considerations to achieve the desired attributes in the final product.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative ai technologies such as large language models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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