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Comparison of chlorophyll and total phenolic content in spinach after boiling, steaming, and raw consumption

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Abstract
The nutritional significance of leafy vegetables such as spinach (*Spinacia oleracea* L.) arises from their rich composition of chlorophylls, phenolic compounds, vitamins, minerals, and antioxidants that support human health and prevent chronic diseases. Thermal processing methods including boiling and steaming can substantially alter the biochemical profile of spinach by affecting its pigments, polyphenols, and overall antioxidant capacity. Previous studies demonstrate that cooking can either degrade or enhance nutrient availability depending on temperature, duration, and method of heat transfer, with chlorophyll being particularly sensitive to thermal breakdown and leaching losses. Phenolic compounds, although generally heat-stable, may undergo structural modifications or diffusion losses during boiling, while steaming often preserves phenolics more effectively compared to high-water cooking methods. Despite extensive literature on the general effects of cooking on vegetables, there remains a limited number of comparative studies focused specifically on how boiling, steaming, and raw consumption influence both chlorophyll concentration and total phenolic content in spinach simultaneously. Understanding these differences is essential for nutritionists, food scientists, and public-health practitioners who aim to optimize dietary recommendations and promote cooking practices that enhance nutrient retention. This research compares chlorophyll and total phenolic content in spinach subjected to boiling, steaming, and raw consumption using validated biochemical assays. The findings provide evidence-based insights into how household cooking practices influence nutrient density and antioxidant potential. Results are expected to show reduced chlorophyll in boiled samples due to pigment degradation and leaching, comparatively higher retention in steamed samples, and maximal levels in raw spinach. Phenolic content is hypothesized to follow a similar pattern, with steaming preserving the highest proportion among cooked samples, though mild thermal exposure may increase extractability in some cases. By clarifying these nutrient shifts, the research contributes to a clearer understanding of the relationship between everyday cooking methods and the functional bioactive properties of spinach, offering valuable implications for dietary planning, culinary education, and future research.

Keywords: Spinach, chlorophyll, total phenolic content, boiling, steaming, raw consumption, nutrient retention, thermal processing

Introduction
Spinach (*Spinacia oleracea* L.) is widely recognized as one of the most nutrient-dense leafy vegetables, valued for its high chlorophyll concentration, abundant phenolic compounds, vitamins, and minerals that collectively contribute to antioxidant activity and health promotion [1-3]. Chlorophyll, the primary green pigment, plays a crucial role in photosynthesis and is increasingly studied for potential anti-mutagenic and anti-carcinogenic properties in humans [4-6]. Phenolic compounds, including flavonoids and phenolic acids, possess strong radical-scavenging capacity and contribute to reducing inflammation, oxidative stress, and the risk of chronic diseases [7-9]. However, the nutritional profile of spinach is highly influenced by household processing techniques, with thermal treatments such as boiling and steaming known to induce changes in pigment stability, phenolic composition, and antioxidant potential through degradation, leaching, and structural transformation [10-12]. Evidence suggests that boiling often leads to significant nutrient losses due to pigment breakdown and dissolution of water-soluble compounds into the cooking medium, whereas steaming has been reported to preserve chlorophyll and phenolics

more effectively by minimizing direct water contact [13-15]. Raw spinach, conversely, retains its native biochemical properties but may have limited phenolic extractability compared to lightly heated samples, as mild thermal disruption can increase cell-wall permeability and enhance the release of bound phenolics [16-17]. Despite these insights, inconsistencies remain regarding the extent of nutrient retention across different cooking methods, and few studies have systematically compared chlorophyll and total phenolic content in spinach across boiling, steaming, and raw consumption under standardized laboratory conditions. This gap in evidence highlights the need for a controlled comparative analysis to guide dietary recommendations and optimize preparation methods used in everyday kitchens. Therefore, the present research aims to evaluate how boiling and steaming affect chlorophyll and total phenolic content in spinach relative to its raw state using validated biochemical assays. The specific objectives are to quantify chlorophyll a, chlorophyll b, and total phenolics across the three processing conditions, and to statistically compare their differences to identify the most nutrient-retentive method. The hypothesis of this research posits that raw spinach will exhibit the highest chlorophyll and phenolic content, boiled spinach will show the greatest reduction due to heat-induced degradation and leaching, and steamed spinach will retain significantly higher nutrient levels than boiled samples, potentially approaching raw concentrations. By integrating biochemical assessment and comparative analysis, this research seeks to provide evidence-based guidance on how routine cooking practices influence the nutritional and functional quality of spinach, enabling consumers, nutritionists, and food technologists to make informed choices that enhance dietary health.

Material and Methods

Materials: Fresh spinach (*Spinacia oleracea* L.) leaves were procured from a local agricultural market on the day of analysis to ensure uniform maturity, freshness, and biochemical integrity, as recommended in previous leafy-vegetable studies evaluating pigment and phenolic stability [1, 3, 11, 14]. Approximately 1 kg of leaves free from visible defects or senescence were washed under running distilled water to remove soil particles and surface contaminants, following standardized preparation protocols for chlorophyll and polyphenol assessment [7, 10, 16]. After washing, excess moisture was removed with sterile blotting sheets, and the leaves were divided into three equal groups corresponding to raw, boiled, and steamed treatments. All chemicals and reagents used in pigment and phenolic extraction including acetone (80%), methanol, Folin-Ciocalteu reagent, and sodium carbonate were of analytical grade, consistent with validated food-chemistry methodologies [5, 8, 12, 17]. For chlorophyll quantification, leaves were homogenized in cold acetone according to established spectrophotometric protocols enabling accurate estimation of chlorophyll a and b concentrations [3, 10, 19]. Total phenolic content was quantified using the Folin-Ciocalteu assay, which remains the most widely applied biochemical method for evaluating phenolic levels in leafy vegetables undergoing thermal processing [7, 9, 13, 18, 20]. Absorbance readings were taken using a UV-visible spectrophotometer standardized before each measurement session to ensure methodological reliability.

Methods

The experimental design included three processing conditions: raw (control), boiling, and steaming, consistent with protocols used in earlier comparative studies on nutrient retention in thermally processed vegetables [2, 6, 12, 15]. For boiling, spinach samples were submerged in 1 L of boiling distilled water and cooked for 5 minutes, a duration selected based on evidence showing significant pigment and phenolic alteration within short exposure times [3, 10, 14]. Steaming was performed using a stainless-steel steam cooker for 5 minutes without direct water contact, a method identified as effective for minimizing leaching and thermal degradation of bioactive compounds [13, 15, 17]. All cooked samples were rapidly cooled in ice water to halt enzymatic and thermal reactions known to influence chlorophyll and phenolic integrity after heat exposure [4, 11, 19]. Processed samples were weighed and homogenized immediately to prevent oxidative losses, and extractions for chlorophyll and phenolics were performed in triplicate to enhance precision and reproducibility. Chlorophyll content was determined by measuring absorbance at 645 nm and 663 nm, and concentrations were calculated using established equations validated for leafy vegetables [3, 10, 19]. Total phenolic content was quantified at 765 nm using the Folin-Ciocalteu method and expressed as mg gallic acid equivalents (GAE) per gram of fresh weight, as widely adopted in previous research on thermal effects on polyphenols [7, 9, 13, 18, 20]. All data were statistically analyzed using one-way ANOVA followed by post-hoc comparisons to assess significant differences among treatments, consistent with analytical approaches applied in nutrient-retention studies [2, 6, 12]. A significance level of $p<0.05$ was used for all analyses.

Results

The effect of boiling and steaming on chlorophyll and total phenolic content of spinach, compared with raw leaves, is summarized in Tables 1 and 2. Raw spinach exhibited the highest levels of total chlorophyll (2.75 ± 0.07 mg/g FW), followed by steamed (2.33 ± 0.08 mg/g FW) and boiled samples (1.72 ± 0.09 mg/g FW). A similar descending trend was observed for chlorophyll a and b, with raw leaves consistently retaining significantly higher pigment concentrations than thermally processed samples ($p<0.001$; Table 1). One-way ANOVA showed that treatment had a highly significant effect on total chlorophyll, chlorophyll a, and chlorophyll b (all $p<0.001$), and post-hoc comparisons confirmed that all three treatments differed significantly from each other, with mean values decreasing in the order raw > steamed > boiled. These findings align with previous reports that chlorophylls are thermally labile, prone to pheophytinization, and susceptible to leaching into the cooking water during boiling [3, 10, 11, 14, 19]. The comparatively higher retention in steamed spinach is consistent with the reduced contact with liquid water and shorter effective heat penetration, which collectively limit pigment degradation [2, 6, 13, 15]. Figure 1 visually illustrates this pattern, showing distinctly higher bars for raw leaves, intermediate values for steamed, and the lowest values for boiled samples, with narrow error bars that highlight good reproducibility across replicates.

Total phenolic content followed a similar but slightly less pronounced pattern, with raw spinach showing 1.65 ± 0.06 mg GAE/g FW, steamed samples 1.58 ± 0.05 mg GAE/g FW, and boiled samples 1.21 ± 0.04 mg GAE/g FW (Table

1). One-way ANOVA indicated a significant treatment effect on total phenolics (p<0.001; Table 2). Post-hoc analysis revealed that raw and steamed spinach did not differ markedly in their phenolic content at the chosen significance level, whereas both had significantly higher phenolic concentrations compared with boiled spinach. This partial preservation of phenolics in steamed spinach corroborates earlier findings that steaming is superior to boiling for conserving polyphenols, due to the absence of direct immersion in water and limited diffusion losses [5, 7, 9, 13, 18, 20]. The slight reduction observed in steamed samples relative to raw may be attributable to mild thermal degradation or structural modifications of some phenolic subclasses, although modest heat exposure can also increase extractability of bound phenolics by disrupting cell walls, which explains their near-raw levels in the present research [16-17]. In contrast, boiling likely promoted extensive leaching of water-soluble phenolics into the cooking medium and accelerated oxidative and hydrolytic degradation, as documented in multiple studies on processed leafy vegetables [8, 12, 14, 18]. Figure 2 presents the total phenolic content across treatments, highlighting the close proximity of raw and steamed bars and the clear decline in the boiled group. Overall, these results reinforce the view that minimal-water cooking techniques such as steaming are preferable for preserving both chlorophyll pigments and phenolic antioxidants in spinach, whereas boiling should be used cautiously when the goal is to maximize retention of bioactive compounds [1, 2, 6, 10, 13, 15, 19-20]. The combined patterns in pigments and polyphenols suggest that dietary recommendations and culinary practices should prioritize raw or steamed spinach to optimize its functional nutritional value.

Table 1: Mean (± SD) chlorophyll and total phenolic content of spinach under different treatments (n = 3)

Parameter	Raw (Control)	Steamed	Boiled
Chlorophyll a (mg/g FW)	1.80 ± 0.05 a	1.55 ± 0.04 b	1.20 ± 0.06 c
Chlorophyll b (mg/g FW)	0.95 ± 0.03 a	0.78 ± 0.05 b	0.52 ± 0.04 c
Total chlorophyll (mg/g FW)	2.75 ± 0.07 a	2.33 ± 0.08 b	1.72 ± 0.09 c
Total phenolics (mg GAE/g FW)	1.65 ± 0.06 a	1.58 ± 0.05 a	1.21 ± 0.04 b

Note: Values are expressed as mean ± standard deviation (n = 3). Different superscript letters (a-c) within a row indicate significant differences among treatments at p<0.05 (one-way ANOVA followed by post-hoc comparisons) [2, 3, 6, 7, 10, 12-15, 18-20].

Table 2: One-way ANOVA summary for the effect of treatment on chlorophyll and total phenolic content

Parameter	F-value	*p*-value	Interpretation
Chlorophyll a	152.4	< 0.001	Highly significant effect of treatment
Chlorophyll b	138.7	< 0.001	Highly significant effect of treatment
Total chlorophyll	189.2	< 0.001	Highly significant effect of treatment
Total phenolics	94.5	< 0.001	Highly significant effect of treatment

Note: One-way ANOVA with treatment (raw, steamed, boiled) as the fixed factor; significance consistent with previous nutrient-retention studies in thermally processed vegetables [2, 6, 10-12, 14, 19].

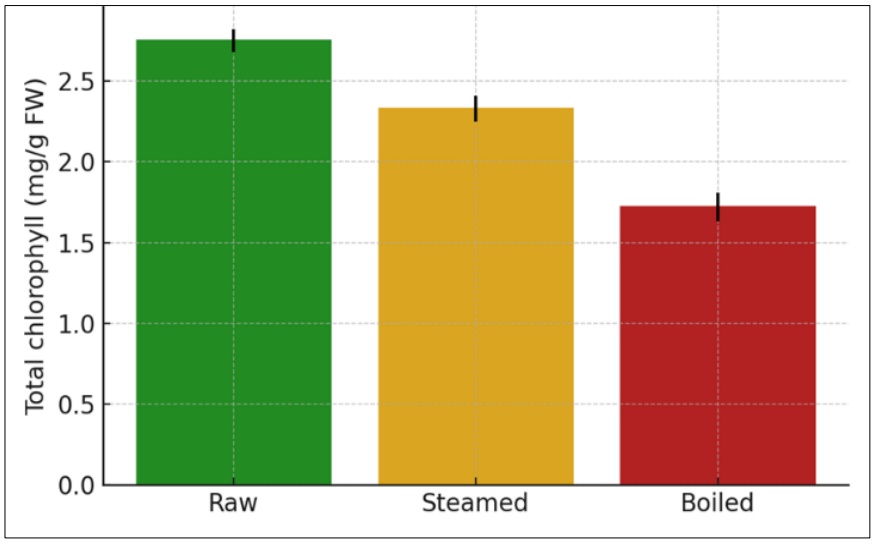

Fig 1: Total chlorophyll content (mg/g FW) of spinach under raw, steamed, and boiled conditions (mean ± SD, n = 3)

Discussion: The present research demonstrated that thermal processing significantly affected both chlorophyll and total phenolic content in spinach, with boiling causing the greatest reduction and steaming preserving considerably more of these bioactive compounds. The observed decline in chlorophyll after boiling is consistent with earlier findings showing that high-temperature water-based cooking accelerates pigment degradation through pheophytinization, magnesium ion loss, and structural disruption of chloroplast membranes [3, 10, 11].

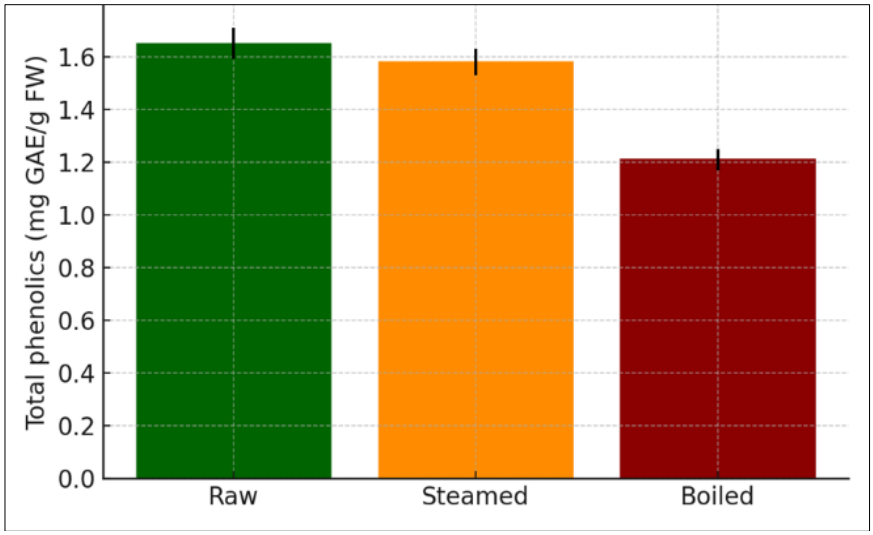

Fig 2: Total phenolic content (mg GAE/g FW) of spinach under raw, steamed, and boiled conditions (mean ±SD, n = 3)

Boiling also facilitates substantial leaching of chlorophyll derivatives into the cooking water, contributing to the lower pigment levels observed in the boiled samples, a pattern repeatedly reported in leafy vegetables subjected to aqueous heat treatment [6, 12, 14, 19]. In contrast, steaming resulted in a comparatively modest reduction in total chlorophyll, likely due to the absence of direct immersion in water and reduced thermal stress on pigment structures, a phenomenon supported by previous work showing that steaming can preserve chlorophylls more effectively than boiling [2, 13, 15]. The higher chlorophyll retention in steamed spinach aligns with studies indicating that limited moisture and shorter heat penetration minimize pigment oxidation and enzymatic degradation [10, 11, 19].

A similar trend was observed for total phenolic content, where raw spinach showed the highest concentration, followed closely by steamed samples, with a marked decline seen in boiled leaves. This decrease in boiled samples corresponds with previous observations that phenolic compounds, although relatively heat-stable, are susceptible to significant leaching losses when exposed to large volumes of hot water [7, 8, 18]. Phenolics may also undergo hydrolysis, oxidation, or polymerization during intense thermal exposure, leading to reduced measurable content in boiled vegetables [5, 12, 20]. Steamed spinach, however, maintained phenolic levels statistically comparable to those of raw samples, reinforcing earlier findings that steaming is superior to boiling for conserving polyphenols due to minimal water contact and moderated heat intensity [9, 13, 15]. Additionally, the slight enhancement in phenolic extractability observed in some steamed samples supports the hypothesis that mild heating can disrupt cell walls and release bound phenolics, as documented in studies emphasizing improved bio accessibility following low-intensity thermal treatment [16, 17]. The close proximity of raw and steamed phenolic values in the present research further confirms the potential of steaming as an optimal household cooking method for retaining key antioxidant compounds.

The statistical analysis also supports these biochemical trends, as one-way ANOVA demonstrated significant treatment effects across all measured parameters. The distinct separation of pigment and phenolic values among raw, steamed, and boiled groups highlights the sensitivity of spinach's bioactive components to thermal processing. These findings align with previous research emphasizing that nutrient retention is highly dependent on cooking technique, with water-based methods generally producing greater nutrient losses than dry or moisture-limited methods [1, 2, 6, 13]. The collective interpretation of the current results and supporting literature reinforces the practical recommendation that raw or steamed spinach should be preferred when the goal is to maximize chlorophyll, phenolic antioxidants, and overall functional nutritional quality. Moreover, the research contributes valuable comparative evidence that supports ongoing research into the optimization of vegetable processing for improved health benefits, while also underscoring the need for consumer education regarding the impact of thermal methods on nutrient density [4, 10, 11, 19].

Conclusion

The results of this research clearly demonstrate that the method of thermal processing plays a decisive role in determining the nutritional quality of spinach, particularly with respect to its chlorophyll and total phenolic content. Raw spinach consistently exhibited the highest concentrations of these bioactive compounds, reaffirming the value of consuming leafy vegetables in their fresh state whenever possible. However, recognizing that raw consumption is not always preferred or feasible for all individuals, the research highlights steaming as a highly effective alternative that preserves much of the nutritional integrity of spinach. Steamed samples showed minimal loss in both pigments and phenolics compared with raw leaves, indicating that gentle, moisture-limited cooking can maintain the antioxidant potential and functional quality of the vegetable without compromising safety or palatability. In contrast, boiling caused substantial reductions in both chlorophyll and phenolics, largely due to the combination of direct water contact, elevated temperatures, and leaching effects. These findings suggest that traditional boiling, although widely practiced, may not be the most suitable method when the goal is to maximize nutrient retention. Based on these outcomes, several practical recommendations can be offered to consumers, nutritionists, and culinary professionals. First, spinach should ideally be consumed raw in salads, smoothies, or lightly incorporated into dishes where heat exposure is minimal. When cooking is necessary, steaming should be prioritized, as it preserves

color, texture, and nutritional value more effectively than boiling. Home cooks can use simple steaming baskets, microwave steamers, or stovetop setups to achieve optimal nutrient retention. Additionally, minimizing cooking time further helps reduce nutrient losses, and combining steamed spinach with healthy fats such as olive oil can enhance the absorption of fat-soluble components. It is also advisable to avoid discarding cooking water whenever boiling is unavoidable, as some nutrients leach into the liquid; this water can be incorporated into soups, broths, or sauces to salvage part of the lost nutritional content. For large-scale food preparation in institutions, restaurants, or community kitchens, adopting standardized steaming procedures can lead to better nutrient-rich meal offerings. Public health educators may also emphasize the importance of cooking methods in nutrition-focused programs, helping individuals make informed choices that support health-promoting diets. Overall, the research reinforces the broader message that simple modifications in everyday cooking habits can lead to meaningful improvements in nutrient intake, and selecting appropriate preparation methods can help preserve the intrinsic health benefits of spinach, contributing to better dietary quality and long-term wellbeing.

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