Journal of Agriculture and Food Research 16 (2024) 101114

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Nutritional, safety and sensory quality evaluation of unleavened flatbread supplemented with thermal and non-thermal processed spinach powder

Muhammad Waseem [a,b], Saeed Akhtar [b], Tahir Mehmood [a], Muhammad Qamar [b], Wisha Saeed [b], Muhammad Younis [b], Saima Perveen [b], Tariq Ismail [b,**], Tuba Esatbeyoglu [c,*]

[a] Department of Food Science and Technology, Faculty of Agriculture & Environment, The Islamia University Bahawalpur, Bahawalpur, 63100, Pakistan
[b] Department of Food Science and Technology, Faculty of Food Science and Nutrition, Bahauddin Zakariya University, Multan, 60800, Pakistan
[c] Department of Molecular Food Chemistry and Food Development, Institute of Food and One Health, Gottfried Wilhelm Leibniz University Hannover, Am Kleinen Felde 30, 30167, Hannover, Germany

ARTICLE INFO

Keywords:
Spinacia oleracea
Leafy vegetable
Microwave processing
Antinutrient
Pesticide
Fortification
Bread
Product development

ABSTRACT

Spinach (*Spinacia oleracea* L.) is a leafy green vegetable that belongs to the family Amaranthus, sub-family Chenopodiaceae. It is famous for its low-calorie content and rich nutritional profile of zinc, folic acid, iron, calcium, magnesium, retinol, and ascorbic acid. In contrast, pesticide residues like imidacloprid, cypermethrin, bifenthrin, chlorpyrifos, and deltamethrin and antinutrients like alkaloids, phytates, tannins, and oxalates are also found in spinach, which inhibit nutrient absorption and also exert deleterious effects in humans. The present study was aimed at determining the technofunctional and nutritional characteristics and improving the consumer safety aspects of dehydrated spinach powder (SP). Spinach was processed to improve its safety characteristics using thermal and non-thermal processing techniques, including microwave heat processing, blanching, acid, and alkali soaking. Findings on the nutrient composition of raw and treated forms suggest SP developed from raw spinach as a promising source of ash (2.9%), fibers (8.19%), proteins (19.1%), Na (97.9 mg/100g), Ca (1304 mg/100g), K (234.2 mg/100g), Fe (41.1 mg/100g), and Zn (14.3 mg/100g). Microwave heating anticipated the highest decline of the content of alkaloids, oxalates, tannins, and phytates by 85, 87, 88 and 89%, respectively. Similarly, microwave heating of SP was found to be more promising in reducing the burden of pesticides such as imidacloprid, cypermethrin, bifenthrin, chlorpyrifos, and deltamethrin by 86, 74, 84, 80 and 78%, respectively. Value-added unleavened flatbreads (chapatis) with 5% SP were observed to have the better color, taste, and textural attributes. The study proposes thermal processing i.e., microwave heat processing in particular as a safer approach to reduce the natural antinutrients and extrinsic toxicants of spinach to a level considerably safer for consumption.

1. Introduction

Spinach (*Spinacia oleracea* L.), a leafy green vegetable belonging to the Chenopodiaceae family, is globally preferred for consumption owing to its notable phytonutrient profile [1]. Foliar of spinach reveals diverse health-promoting phytochemicals such as lutein, zeaxanthin, β-carotene, chlorophyll, flavonoids, and ascorbic acid, exhibiting appreciable biological and antioxidant activities [2,3]. Spinach is also known for its unique dietary fibers, vitamins (i.e., riboflavin and pyridoxine), and mineral composition consisting of zinc, iron, manganese, copper, calcium, potassium, phosphorus, and magnesium, which contribute to promoting health and providing nutrition [4]. Earlier studies indicate spinach as a viable source of phenolics, spinacetin, patuletin, folates, carotenoids, and iron, which elicit ameliorative health effects against numerous maladies, including eye disorders like macular degeneration, neural tube defects, cancer, nutritional anemia, hypercholesteremia, hyperlipidemia, hyperglycemia, cardiovascular maladies, inflammation, and obesity [5,6]. Spinach could be used in a multitude of food

* Corresponding author.
** Corresponding author.
E-mail addresses: mwaseem@iub.edu.pk (M. Waseem), saeedbzu@yahoo.com (S. Akhtar), tahiraridian@gmail.com (T. Mehmood), muhammad.qamar44@gmail.com (M. Qamar), wishasaeed1@gmail.com (W. Saeed), younismian0@gmail.com (M. Younis), saimaparveen431@gmail.com (S. Perveen), tariqismail@bzu.edu.pk (T. Ismail), esatbeyoglu@lw.uni-hannover.de (T. Esatbeyoglu).

https://doi.org/10.1016/j.jafr.2024.101114
Received 3 January 2024; Received in revised form 27 February 2024; Accepted 15 March 2024
Available online 16 March 2024

applications, such as fresh, dehydrated powders, curries, gravies, salads, fabricated or processed foods, baked goods, nutritional supplements, weight loss diets, and functional drinks [7–9].

Antinutrients are recognized to interfere with the metabolic fate of proteins and minerals and thereby affect their bioavailability. Green leafy vegetables belonging to Brassica family are supposed to carry magnitudes of health affecting antinutrients such as alkaloids, tannins, phytates, and oxalates. Complications of these toxic antinutrients are not only limited to the interference with the minerals but are also linked with the several health complications in humans such as kidney stones and renal failure. However, use of thermal processing like microwave heat processing could be helpful in reducing the burden of antinutrients in human health [10]. Similarly, injudicious use of pesticides in developing countries on fruits and vegetables for the management of insects and pests has made consumers at risks of possible toxicity associated with higher residual levels of pesticides. According to the global report by the Environmental Protection Agency (EPA), about 3 billion kg of pesticides are being used worldwide, of which 20 and 80% are used by developing and developed countries, respectively [11]. Consumption of pesticide residue-laden vegetables may have deleterious consequences for humans in the form of neurological disorders, reproduction problems, birth defects, miscarriage in women, memory loss, immunological and behavioral disorders, cancers, hypersensitivities, spasms, paralysis, and allergic responses [12,13]. The maximum residual limit (MRL) of several pesticides, including bifenthrin, cypermethrin, imidacloprid, deltamethrin, and chlorpyrifos, for leafy vegetables has been enlisted by Codex Alimentarius in a range between 0.4 and 2 ppm [14]. Retrospective studies by Refs. [15,16] have elucidated the presence of pesticides like cypermethrin, chlorpyrifos, dimethoate, monocrotophos, and methamidophos in spinach beyond their maximum allowable limits. Given the existence of toxicants including antinutrients and pesticides in vegetables and their health hazards, the scientific community is interested in reducing the growing illness burden among high-risk consumers [10].

Likewise, thermal (blanching), non-thermal (acid soaking, alkali soaking), chemical (acetic acid, citric acid soaking), physical (peeling, grating), and biological (fermentation) techniques have been used to alleviate toxicant loads in fresh produce at domestic and industrial levels [17,18]. Previous studies on the thermal processing of vegetables have revealed an appreciable reduction in toxicants, which could be attributed to heat degradability, strength and extent of ionic bonding between toxicants and food constituents, and extent of solubility in water [18,19]. A recent study has also reported the positive effects of microwave heat processing, alkali soaking, acid soaking, and blanching in reducing the pesticide and antinutrient load in leafy greens to permissible or safer limits for consumption [10].

A plausible amount of data is available that validates raw spinach as a promising source of nutrients alongside a range of nutrients inhibitors. However, limited information is available on cost-effective, approachable and practical measures that may be deployed at the household and industrial levels to mitigate the burden of intrinsic toxicants i.e., antinutrients (alkaloids, tannins, phytates and oxalates) and extrinsic toxicants i.e., pesticide residues in spinach. The present study was therefore planned to evaluate the effect of thermal and non-thermal detoxification techniques including microwave heat processing, blanching, acid and alkali soaking in reducing the intrinsic and extrinsic toxicants in spinach and to define the maximum level of supplementation of processed spinach with the least loss in sensory quality of unleavened flatbread or chapatti.

2. Material and methods

2.1. Raw materials, reagents and chemicals

Approximately 15 kg of fresh spinach leaves were obtained from the field area of the Faculty of Food Science and Nutrition, Bahauddin Zakariya University, Multan, Pakistan. Fresh, insect- and pest-free spinach leaves were preliminary sorted, graded for color, wilting, and physical quality, washed with clean distilled water, and uniformly chopped using a stainless-steel knife. All analytical and HPLC-grade reagents and chemicals used in the technofunctional, proximate, mineral, and antinutrient analyses and product development were procured from Merck (Darmstadt, Germany) and Sigma-Aldrich (Steinheim, Germany). Analytical standards used in the study for elemental analysis (i.e., Ca, K, Na, Zn, and Fe), antinutrient analysis (oxalates, alkaloids, tannins, and phytates), and pesticide analysis (i.e., cypermethrin, imidacloprid, chlorpyriphos, bifenthrin, and deltamethrin) were purchased from Sigma-Aldrich.

2.2. Processing of raw spinach

Microwave heat processing: Freshly sorted, graded, and washed 3 kg of spinach leaves were microwave heat processed for detoxification purposes at 1.1 kW for 2 min.

Blanching: Evenly chopped fresh leaves of spinach were hot water (1:10 *w/v*) blanched at 100 ± 2 °C for 1 min. Extraneous surface water was drained off and blanched spinach leaves were pre-dried on clean muslin cloth.

Acid soaking and alkali soaking: Acid soaking and alkali soaking of spinach leaves were performed as outlined by Amin et al. [20]. About 2 kg of shredded spinach leaves were soaked for 16–18 h in 2 M hydrochloric acid. A mixture of 1.5% sodium bicarbonate, 0.5% sodium bicarbonate, and 1% sodium bicarbonate solutions was used for the alkali soaking of spinach leaves.

2.3. Raw and processed spinach powder preparation

Raw and processed spinach leaves were spread over the nylon trays (mesh size = 0.186 × 0.186 m^2) and dried at 45 °C for 10–12 h using a cabinet dryer (Pamico Tech, Pakistan, Faisalabad) to a final moisture level of 8–10%. Subsequently, dehydrated raw and processed spinach leaves were converted into fine powder (i.e., 72 mm) in a heavy-duty grinder (Pamico Tech, Pakistan, Faisalabad). The raw and processed spinach powder samples were kept in vacuum jars for further use in the study [21].

2.4. Technofunctional characteristics of raw and treated spinach powders

2.4.1. Bulk density and rehydration ratio

All raw and processed spinach powders were analyzed for bulk density by following the protocol as outlined by Shafi et al. [22]. The rehydration ratio of control and treated samples of spinach was estimated as documented by Yu et al. [23]. Precisely measured 5 g of each spinach leaf sample was homogenized in 50 mL of clean distilled water and kept at room temperature for 30 min, filtered, and precisely weighed. The rehydration ratios of all spinach samples were estimated using the following formula:

$$Rehydration\ Ratio = \frac{Wt.\ of\ drained\ material\ (W1)\ (g)}{Wt.\ of\ dehydrated\ residues\ (W2)\ (g)} \quad (I)$$

2.4.2. Water absorption index (WAI)

The water absorption index of raw and processed spinach powder samples was determined by following the methods as outlined by Sharma et al. [24]. About 12.5 g of each sample was transferred into a conical flask already holding 15 mL of distilled water and mixed for 30 min. Thereafter, the mixture of each sample was centrifuged (Hermle Z236K, Wehingen, Germany) for 20 min at 4300 rpm, and the supernatant was dehydrated at 105 ± 5 °C for 24 h using a drying oven (Memmert UNB 200, Schwabach, Germany). The amount of water absorbed per gram of sample was expressed as WAI.

2.4.3. Water solubility index (WSI)

Accurately measured 1 g of each spinach powder sample was blended in distilled water and heated for 3 min at 80 ± 3 °C using water bath (Memmert, Schwabach, Germany). Subsequently, the contents were mixed in centrifuge machine at 4300 rpm, for 15 min followed by oven-drying at 70 ± 2 °C, for 8 h [25]. WSI of all samples was measured using formula mentioned hereunder:

$$\text{Water solubility index } (\%) = \frac{W_1\ (g) - W_2\ (g)}{W\ (g)} \times 100 \quad \text{(II)}$$

W = Weight of each dehydrated spinach sample.
W1 = Weight of each oven dried spinach sample with Petri dish.
W2 = Petri dish weight.

2.4.4. Swelling power (Sp)

Swelling power of each spinach powder sample was estimated according to the protocol as adopted by Gani et al. [26]. Precisely measured 3.0 g of each spinach powder sample was thoroughly mixed in 30 mL distilled water and heat treated for 30 min at 70 ± 5 °C using water bath. Thereafter, each sample was centrifuged for 10 min at 1500 rpm. Supernatants of all mixtures were collected, and solid residues deposited in falcon tubes were accurately measured for weight. S*p* of all samples was estimated by the formula given below:

$$\text{Swelling power} = \frac{\text{Solid residues weight } (g)}{\text{Initial sample powder weight } (g)} \quad \text{(III)}$$

2.4.5. Hygroscopicity (Hg)

Hygroscopicity values of each spinach powder sample was assessed using the method as documented by Costa et al. [27]. About 10 g of each raw and processed spinach powder sample was taken in desiccator containing sodium sulphate (Na_2SO_4) to maintain relative humidity up to 80%. On completion of duration, the samples were taken off and accurately weighed. Hence, the moisture absorbed by samples (g) per 100 g of dry solids was expressed as hygroscopicity.

2.5. Nutritional composition and minerals profiling

The nutritional profile was evaluated according to the methods of AOAC [28]. Moisture (925.10), ash (923.03), protein (920.87), fat (920.85), and fiber (32-10) were determined using standard analytical methods., and nitrogen free extracts (NFE) in all spinach powder samples. However, carbohydrates were estimated by using the formula as:

Carbohydrates (NFE) (g/100g) = (100) – % (ash + moisture + fat + protein + fiber)

Inorganic elements including Ca, Na, K, Fe and Zn in each spinach powder sample were determined in accordance with the standard protocol as laid down in official protocols of American Association of Analytical Chemists (AOAC) [28] (No. 40–70.01) using the flame photometer (410, Sherwood Scientific Ltd, Cambridge, UK) and atomic absorption spectrophotometer (Thermo Scientific iCE 3000 series, Waltham, MA), respectively.

2.6. Antinutrients contents determination

2.6.1. Alkaloids determination

About 5 g of each spinach powder sample was blended in 10%, 50 mL ethanolic solution of acetic acid and given a stay time of 4 h. Thereafter, initial filtration (Whatman filter paper no. 41) was performed to obtain 1/4th of the initial content. Alkaloids were precipitated by titrating the filtered content with conc. ammonium hydroxide (NH_4OH). A second washing was given to the alkaloid precipitates using 1% ammonium hydroxide solution and filtered again. Precipitates on the surface of filter paper were dried in hot air oven at 60 ± 5 °C for 30 min. Dried alkaloid precipitates accompanied with the filter paper were weighed [29].

$$\text{Alkaloids } (mg/100g) = \frac{W2 - W1\ (g)}{Ws\ (g)} \times \frac{100}{1} \quad \text{(V)}$$

Ws = Weight of raw and processed spinach samples.
W1 = Weight of empty filter paper.
W2 = Weight of filter paper accompanied with the precipitates.

2.6.2. Determination of oxalates contents

A protocol of Amin et al. [20] was followed to estimate oxalates in raw and processed forms of spinach powder. Precisely measured 1.0 g of raw and processed spinach powder samples were taken and digested in 6 M, 10 mL hydrochloric acid (HCl), and 190 mL distilled water for 1 h and centrifuged for 10 min at 2000 rpm. Fifty milliliters of supernatant were taken and heated using a hot plate (LMS-1003; Daihan Labtech Co. Ltd., Namyangju, South Korea) to concentrate up to 25 mL, then rested for 15 min. The concentrate was filtered to obtain brown precipitates on the filter paper. Concentrated ammonia solution was poured over the precipitates for pH adjustment to a faint yellow or salmon pink end color. Subsequently, a 5%, 10 mL calcium chloride ($CaCl_2$) solution was added to precipitate the oxalates and filtered. Thereafter, titration was performed using 0.05 M potassium permanganate ($KMnO_4$) to observe the pink color endpoint. Oxalates were estimated by following the formula given below;

Oxalates (mg/100g) = (Titer value) x (0.1125)

2.6.3. Determination of tannins contents

Tannin contents of all samples were estimated in accordance with the method as outlined by Amin et al. [20]. About 0.5 g of each spinach powder sample was mixed in 7.5 mL of distilled water and boiled for 30 min. The reaction mixture was cooled and centrifuged for 20 min at 2000 rpm. Now, 1 mL of supernatant was mixed with 1 mL of 7.5% sodium carbonate and 5 mL of Folin-Denis reagent to adjust the final volume up to 10 mL and allowed to stay for 30 min. Absorbances of control and processed dehydrated spinach samples at 700 nm were measured using a spectrophotometer (UV–Vis 3000, Darmstadt, Germany) against tannic acid standards (i.e., 10–100 ppm) and reagent blank to estimate tannin content (mg/100 g).

2.6.4. Determination of phytates contents

Accurately weighed, 1.0 g of each dehydrated spinach powder sample was mixed in 10.0 mL of 0.2 N HCl with continual stirring for 30 min. Thereafter, 0.5 mL of extract was transferred to 1 mL of ammonium iron (III) sulphate solution, subjected to boiling for 30 min, and rested for 20 min. The admixture was centrifuged at 3000 rpm for 30 min. Thereafter, 1 mL of the centrifuged supernatant was shifted to a volumetric flask containing 1.5 mL of 2,2′-bipyridine solution (0.25 g each of thioglycolic acid and 2,2′-bipyridine were mixed in distilled water up to a total volume of 25 mL). Spectrophotometric absorbance of all samples was recorded at 519 nm against phytate-phosphorous (100–1000 mg/L) as a standard and reagent blank [30].

2.7. Pesticide residues extraction, clean-up process and determination

Each sample of raw and processed spinach powder was homogeneously grinded and converted to a paste for pesticide residues extraction using HPLC grade ethyl acetate. About 50 g paste was amalgamated with 10 mL saturated sodium chloride and 20 g anhydrous sodium sulphate in conical flask (250 mL). Following mixing, 75 mL ethyl acetate was added into the mixture and stirred for an hour at 240 rpm using orbital shaker (MaxQ™ 4000, Thermo Scientific, Waltham, MA, USA) and filtered. The extracted samples were stored at −80 ± 2 °C for further analysis.

Clean-up of the HPLC column was done in accordance with the method as documented by EL-Saeid and Selim [31] with slight modifications. A glass wool layer covered with anhydrous sodium sulphate was placed at the bottom of chromatographic column followed by silica gel and charcoal mixture i.e., 7:5 (*w/w*) layer. The flow rate was adjusted at 1 mL/min and column was eluted using 50 mL mixture of HPLC grade methanol:acetone (7:3 *v/v*) for clean-up. Eluate was concentrated up to 1 mL using a rotary evaporator at 40 °C and collected in glass vials. Eluate was washed with methanol followed by controlled nitrogen gas fluxing under controlled pressure in petri plates for removal of impurities.

Imidacloprid, cypermethrin, bifenthrin, chlorpyrifos, and deltamethrin in all samples of spinach were estimated on HPLC using UV–visible detector (PerkinElmer, Series 200, Waltham, MS, USA) by following the method of Abdullah et al. [32]. Precisely measured 20 μL homogeneous slurry of each sample was injected into HPLC C18 column (Supelco, Bellifonte, PA, USA; 250 mm × 4.6 mm i.d.) at 30 °C using an auto-sampler. Methanol:water (45:55, *v/v*) were used as mobile phase and flow rate was adjusted 1 mL/min. Sample run time was adjusted at 20 min at 254 nm against the standard curves of 2, 4, 6, 8 and 10 ppm and reagent blank.

2.8. Production and sensory evaluation of spinach powder supplemented flatbreads

Precisely weighed 50 g dough was prepared using whole wheat flour and clean water. Each dough was converted into unleavened flatbread and baked for 2–3 min on both sides at 200 ± 5 °C onto a cast iron plate. Organoleptic evaluation of raw (T_0) and processed chapatis prepared with the addition of 2.5–10% microwave heat treated spinach powder) were performed by 20 sensory experts. Sensory scores were assigned in accordance with 9-point hedonic scale for physical appearance, folding ability, color, texture, taste and overall acceptability.

2.9. Statistical analysis

All experiments were performed in twice as two different experiments. Findings on the technofunctional, physico-chemical, intrinsic and extrinsic toxicants and sensory parameters for raw and microwaved spinach powder substituted flatbreads were recorded as means ± S.E. using analysis of variance (ANOVA) technique on Statistix 8.1 (Tallahassee, FL, USA). Least significant difference (LSD) test was performed at 5% confidence interval to assess significant differences among treatment means.

3. Result and discussion

3.1. Technofunctional properties of raw and processed spinach powders

Technofunctional characteristics of raw and treated (microwave heated, blanched, acid soaked, alkali soaked) spinach powders were evaluated, but comparatively raw spinach powder was recorded to exhibit significantly ($p \leq 0.05$) higher WSI, WAI, Sp, and Hg, i.e., 4.3%, 3.3 g/g, 7.1 g/g, and 5.7%, respectively (Fig. 1). Whereas, on comparison of efficiency of different processing treatments, microwave processing reported significantly ($p \leq 0.05$) higher mean values for WSI, WAI, and Sp i.e., 4.1%, 3.2 g/g, and 6.7 g/g, respectively. A study by Suriya et al. [33] revealed comparable findings for WSI, WAC, and SP in blanched elephant foot yam flour wherein, blanching for 5 min resulted in significant decrease of WSI, and SP from 16.8 to 8.5% (50% decrease), and 8.5 to 6.2% (27% decrease), respectively. A decrease in WSI and SP may be attributed to structural disorganization of carbohydrates such as starch and thermal degradation [33]. However, the results for WAC of

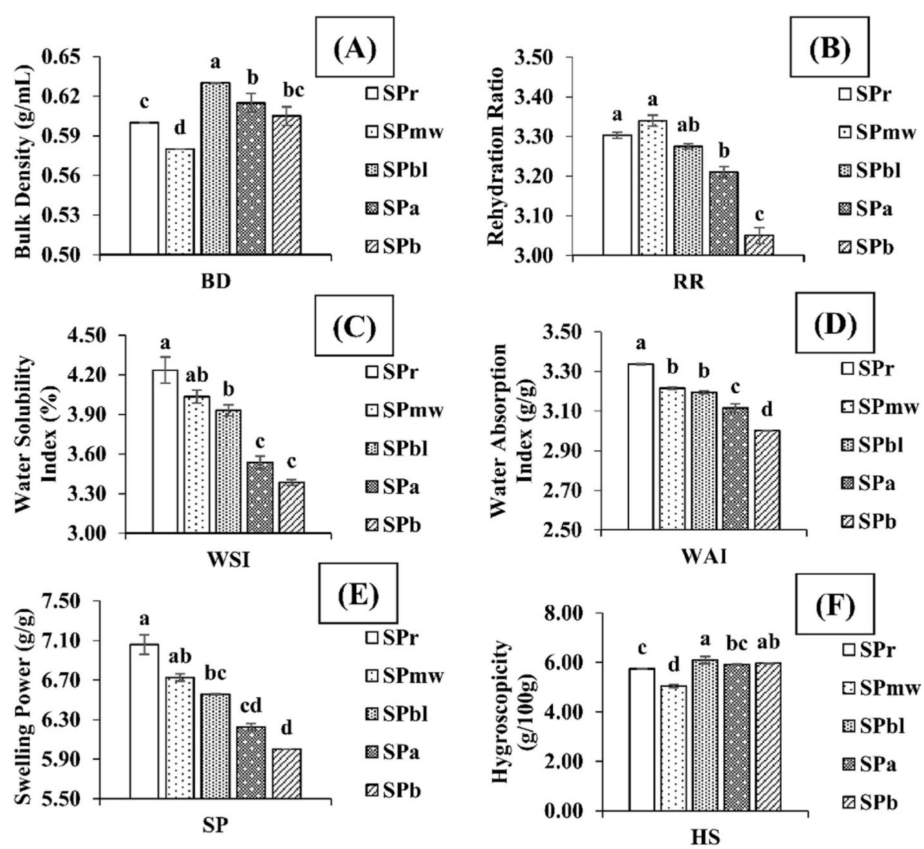

Fig. 1. Technofunctional attributes of raw and treated spinach powder
SPr = Raw spinach powder, SPmw = Microwave treated spinach powder, SPbl = Blanched spinach powder, SPa = Acid treated spinach powder, SPb = Base (alkali) treated spinach powder.

blanched elephant yam flour showed an average increase of 3.2–3.4%. Results of present study were compared with elephant foot yam flour because a meager amount of data is available on techno-functional attributes of spinach/green leafy vegetables. The variation in WSI, SP, and WAC could be linked with the differences in heat stability of macro-nutrients such as dietary proteins and fibers available in vegetable flours. An increase in the water absorption capacity of flours could be associated with the flours' ability to solubilize, higher magnitudes of carbohydrates and proteins, higher concentrations of amylose, and crystalline starch damage [33].

3.2. Nutritional composition of raw and processed spinach powders

Proximate analysis of raw and treated spinach powder was performed and results are presented in Table 1. The results for the nutritional composition are in agreement with the findings of Galla et al. [34] and Khan et al. [35] wherein, the authors reported appreciable amounts of moisture, ash, proteins, and fiber contents i.e., 4.8–8.6, 10–19, 21–28, and 8.8–11.3%, respectively. Minimal differences observed in nutritional components including ash, protein and fiber in tested plant foods could be attributed to variation in cultivars, agro-climatic conditions, environmental stress, type of soil, and climate [36]. In present analysis, after processing of the raw SP, the highest ash, protein, and fiber were recorded in acid soaked, alkali soaked, and microwave heated versions i. e., 3.8, 19.0, and 10.1%, respectively. In preview of the rich nutritional profile of raw SP, processing treatments significantly ($p \leq 0.05$) improved ash i.e., from 3.0 to 3.8% and dietary fibers i.e., from 8.2 to 10.1%, while a significant decline was noticed in crude protein from 19.2 to 17.1% (Table 1). The decrease of proteins could be linked with the thermal degradation of nitrogen-based components on heat processing of SP. In agreement to our findings, Chib et al. [37] revealed a significant decrease in protein contents of *Amaranthus* leaves from 3.2 to 2.9% and 3.2 to 3% on blanching for 6 min and microwave heat processing for 45 s, respectively. However, the study also elucidated a significant increase in fiber contents of the Amaranthus leaves from 1.9 to 2% and 1.9–2.2% under same heat processing conditions. Similarly, in another study by Suriya et al. [33], authors revealed a reduction in protein contents of elephant foot yam flours from 10 to 8% on blanching for 5 min. Another study by Tafu and Jideani [36] reported comparable findings for nutritional components wherein the researchers elucidated significant decrease in ash, protein, and fiber contents from 29 to 6%, 12 to 6%, and 0.5 to 0.2% on microwave heat processing of moringa leaf powder.

3.3. Mineral composition of raw and processed spinach powders

Leafy green vegetables are appreciated for their micronutrients which embodies vital role against several health challenges and micronutrient malnutrition. Mineral contents of raw SP samples were significantly ($p \leq 0.05$) higher as compared to the processed versions of SP (Table 2). Among processed SP powder the highest Na contents (96.0 mg/100g) were recorded in in blanched, Ca contents (1301 mg/100g) in acid-soaked whereas K (231.7 mg/100g), Fe (39.0 mg/100g) and Zn (13.0 mg/100g) contents in microwave heat-treated. Comparison of mean concentrations of minerals shown non-significant ($p \leq 0.05$) effect of processing treatments on K contents of processed SP excepting all other minerals wherein significant ($p \leq 0.05$) change was observed against each processing treatment. Different processing methods anticipated a decline in minerals levels of SP which could be linked with leaching of soluble fractions of minerals, cellular destruction of plant tissues on heating, and treatment with acids and alkalis.

Earlier studies have reported the dehydrated spinach powders as enriched carriers of minerals such as sodium (3279 mg/100g), calcium (1019–1336 mg/100g), potassium (336–1094 mg/100g) and microminerals i.e. zinc and iron 5 and 30–69 mg/100g, respectively [34, 35]. However, retrospective studies have validated the decrease in Fe contents of Amaranthus leaves from 53 to 42 and 53.2 to 51.4 mg/100g after blanching for 6 min and microwave heating for 45 s, respectively [37]. A recent study by Tafu and Jideani [36] elucidated a significant reduction in mineral elements on microwave heat processing of moringa leaf powder samples wherein, the Na, Ca, K, Zn and Fe contents were decreased from 3.8 to 2.9, 41 to 38.3, 14 to 7.2, 0.4 to 0.03, and 6.1 to 2.5 mg/100g, respectively.

Table 2
Mineral composition of raw and processed forms of spinach powder (mg/100g).

. Treatments	Na	Ca	K	Fe	Zn
Raw SP	97.99 ± 0.70^{a}	1304 ± 0.04^{a}	234.2 ± 0.10^{a}	41.06 ± 0.34^{a}	14.26 ± 0.46^{a}
Microwave Heating	93.05 ± 0.04^{cd}	1297 ± 1.2^{b}	231.7 ± 0.46^{ab}	39.01 ± 0.42^{b}	13.02 ± 0.06^{ab}
Blanching	96.04 ± 0.28^{ab}	1291 ± 0.71^{c}	220.5 ± 0.86^{cd}	37.40 ± 0.07^{cd}	12.00 ± 0.11^{bc}
Acid Soaking	90.82 ± 0.27^{d}	1301 ± 0.71^{ab}	214.5 ± 0.73^{d}	38.30 ± 0.07^{bc}	11.42 ± 0.69^{bc}
Alkali Soaking	93.86 ± 0.79^{bc}	1293 ± 1.77^{c}	223.5 ± 3.37^{bc}	36.40 ± 0.01^{d}	10.73 ± 0.32^{c}

Means having similar letters in a column are statistically non–significant at $p \leq 0.05$, Mean ± S.E. (n = 2). SP, Spinach powder.

Table 3
Antinutrient contents of raw and processed spinach powder (mg/100g).

Treatments	Alkaloids	Oxalates	Tannins	Phytates
Raw SP	456.5 ± 0.90^{a}	954.6 ± 1.19^{a}	383.1 ± 0.73^{a}	285.9 ± 0.48^{a}
Microwave Heating	64.98 ± 2.13^{c}	118.1 ± 3.98^{e}	44.19 ± 1.77^{e}	28.86 ± 0.14^{d}
% Reduction	*85*	*87*	*88*	*89*
Blanching	84.98 ± 3.52^{b}	219.9 ± 2.78^{d}	63.69 ± 0.71^{d}	77.51 ± 1.36^{c}
% Reduction	*85*	*77*	*83*	*73*
Acid Soaking	91.97 ± 1.39^{b}	243.0 ± 3.18^{c}	118.2 ± 1.06^{c}	84.05 ± 2.72^{c}
% Reduction	*80*	*75*	*69*	*70*
Alkali Soaking	94.78 ± 0.86^{b}	331.9 ± 3.98^{b}	139.2 ± 1.77^{b}	96.74 ± 1.36^{b}
% Reduction	*79*	*65*	*63*	*66*

Means having similar letters in a column are statistically non–significant at $p \leq 0.05$, Mean ± S.E. (n = 2). SP, Spinach powder.

Table 1
Nutritional composition of raw and processed forms of spinach powder (g/100g).

Treatments	Moisture	Ash	Protein	Fat	Fiber	Carbohydrates	Caloric values (Kcal/100g)
Raw SP	8.26 ± 0.11^{a}	2.99 ± 0.01^{d}	19.18 ± 0.04^{a}	1.04 ± 0.01^{bc}	8.19 ± 0.02^{cd}	60.34 ± 0.01^{c}	329.3 ± 0.01^{a}
Microwave Heating	7.01 ± 0.07^{cd}	3.26 ± 0.02^{c}	18.11 ± 0.07^{b}	1.15 ± 0.04^{a}	10.11 ± 0.07^{a}	60.27 ± 0.08^{c}	325.76 ± 0.28^{b}
Blanching	7.62 ± 0.02^{c}	3.67 ± 0.01^{b}	17.57 ± 0.05^{c}	0.94 ± 0.02^{c}	8.05 ± 0.04^{d}	62.14 ± 0.07^{a}	329.1 ± 0.24^{a}
Acid Soaking	8.23 ± 0.01^{b}	3.85 ± 0.04^{a}	17.05 ± 0.04^{d}	0.77 ± 0.01^{d}	8.65 ± 0.04^{b}	61.45 ± 0.04^{b}	322.5 ± 0.07^{c}
Alkali Soaking	8.06 ± 0.04^{bc}	3.15 ± 0.04^{c}	19.00 ± 0.00^{a}	1.12 ± 0.02^{ab}	8.36 ± 0.02^{c}	60.31 ± 0.18^{c}	329.3 ± 0.48^{a}

Means having similar letters in a column are statistically non–significant at $p \leq 0.05$, Mean ± S.E. (n = 2). SP, Spinach powder.

3.4. Antinutrients in raw and processed spinach powders

The results presented in Table 3 shows that higher magnitudes of antinutrients including alkaloids (456.5 mg/100g), oxalates (954.6 mg/100g), tannins (383.1 mg/100g) and phytates (285.9 mg/100g) were recorded in raw SP when compared with the processed variants. The study observed a positive effect of processing techniques in reducing antinutrients contents of the spinach powder. Among the thermal and non-thermal processing treatments, the microwave heating presented the highest % reduction in alkaloids followed by oxalates, tannins, and phytates as 85, 87, 88 and 89%, respectively (Table 3).

Earlier studies evidently reported the positive role of thermal and non-thermal processing techniques in mitigating the ever-increasing burden of toxic antinutrients in green leafy vegetables [38]. In a similar perspective, a study by Wang et al. [39] reported significant reduction of soluble oxalates in spinach to an extent of 28 and 73% on blanching at 60 and 100°C for 3 s and 3 min, respectively. Likewise, another study by Akhtar et al. [40] revealed significant decline in soluble oxalates contents from 50 to 66% in spinach on cooking till softening of the leaves for 12–15 min. A study by Shimada [41] exhibited 72% decline in soluble oxalates in spinach on hot water soaking of spinach at 80 °C for 1 h.

A study by Chib et al. [37] on *Amaranthus* leaves reported 33 and 30% reduction in oxalates on microwave heating and blanching for 45 s and 6 min, respectively. Likewise, another study by Kumoro et al. [42] studied the effect of alkali soaking (i.e., sodium bicarbonate solution 2% for 20 min) on the oxalates contents of giant taro leaves and revealed 85% reduction in oxalates contents from 471.5 to 67 mg/100g. The reduction in antinutrient contents of leafy vegetables such as spinach and giant taro could be linked with the combined effects of leaching, thermal degradation and solubility properties of oxalates during thermal and non-thermal processing [42]. Earlier literature has validated the effectiveness of the microwave heat processing as a viable technique to eliminate antinutrients in different food commodities due to its higher thermal degradation ability [43]. Our results are in close agreement with the literature [43–45] wherein the microwave heat processing at 800–900 W for 2.5–8 min resulted in 100% elimination of total oxalates in red velvet bean, 96% reduction in horse chestnut and 61% reduction in legume horse gram, respectively. Microwave heat processing has also been recommended as an economical alternate to mitigate the antinutrients burden in vegetables.

3.5. Pesticide residues in raw and processed spinach powders

The data on pesticides residues in raw and processed SP samples revealed raw SP to hold significantly ($p \leq 0.05$) higher concentrations of imidacloprid (2.44 ppm), cypermethrin (0.99 ppm), bifenthrin (0.93 ppm), chlorpyrifos (2.19 ppm) and deltamethrin (2.30 ppm) (Table 4). All processing treatments significantly reduced the residues of all pesticides in SP. When compared, microwave heat processing stands as the most effective technique in reducing the loads of imidacloprid (86% reduction), cypermethrin (74% reduction), bifenthrin (84% reduction), chlorpyrifos (80% reduction) and deltamethrin (78% reduction), followed by blanching, acid soaking and alkali soaking. Earlier studies have documented notable effects of acid and alkali soaking, washing, microwave heat processing, blanching and sterilization in mitigating the residues of pesticides in spinach. Bonnechère et al. [46]and Zhao et al. [47] showed that microwave heat processing (i.e., 700 W, 4 min) of jujube fruits resulted in a notable reduction of chlorpyrifos and bifenthrin contents from 66 to 93%. Alkali and water soaking of spinach for 5–30 min reported to cause a significant decline in chlorpyrifos from 19 to 37% and 4–17%, bifenthrin 18–39% and 4–35%, and cypermethrin 7–13% and 51–66%, imidacloprid 9–34% and 29–46%, respectively [48]. Park et al. [49] elucidated 78–94% reduction in pesticides residues of spinach on blanching and washing for 1–5 min.

Comparable findings for pesticide residues were reported by Amir et al. [13] wherein, the authors anticipated 10% acetic acid soaking as the best treatment among all other washing solutions (i.e., citric acid, acetic acid, ginger, garlic extracts) as it reduced the endosulfan, deltamethrin, chlorpyrifos and cypermethrin contents by 76–80%, 53–70%, 94% and 83–90%, respectively. Chung [50] and Nguyen et al. [51] reported spinach blanching (88°C for 5 min) to cause reduction in deltamethrin, propamocarb and iprodione contents by 47, 72 and 58%, respectively. Bonnechère et al. [46] reported the reduction of five pesticides in spinach on microwave heating, blanching and soaking by 39, and 70, 50%, respectively. Siddique et al. [52] exhibited sonolytic ozonation of spinach for 10 min wherein the acetamiprid, imidacloprid, and thiacloprid residues were decreased by 92–100%. The reduction in pesticides residues of the vegetables on thermal and non-thermal processing could be attributed to acid hydrolysis, solubility, leaching, thermal degradation and dissolution of cuticular waxy layer and nature of tissues [52,53]. Lower reduction of pesticide residues on processing via acid and alkali treatment could be linked to the pH of soaking solution, differences in vapor pressure and lower water solubility of pesticides [19,48].

3.6. Effect of microwave heat treated spinach powder supplementation on sensory attributes of unleavened flatbread

Dehydrated spinach like many other vegetables are ranked as value added edible goods for their perspective utilization as a food or food ingredient. The results for organoleptic analysis of microwave heat treated SP supplemented chapatis are given in Fig. 2. Highest sensory scores for color, taste, texture and overall acceptability were recorded in control i.e., 8.2, 8.2, 8.1, and 8.1, respectively (Fig. 2). However, among treatments groups (i.e., T_1 – T_4), T_3 anticipated highest sensory scores for color, taste, texture and overall acceptability i.e., 7.4, 7.4, 7.3 and 7.5, respectively. The highest color and taste scores were assigned to

Table 4
Pesticide residues in raw and processed spinach powders (ppm).

Treatments	Imidacloprid	Cypermethrin	Bifenthrin	Chlorpyrifos	Deltamethrin
Raw SP	2.44 ± 0.01^a	0.99 ± 0.01^a	0.93 ± 0.02^a	2.19 ± 0.03^a	2.30 ± 0.02^a
Microwave heating	0.34 ± 0.01^e	0.25 ± 0.01^d	0.16 ± 0.01^e	0.45 ± 0.02^e	0.51 ± 0.02^e
% Reduction	*86*	*74*	*84*	*80*	*78*
Blanching	0.68 ± 0.02^d	0.29 ± 0.01^d	0.25 ± 0.02^d	0.62 ± 0.02^d	0.78 ± 0.02^d
% Reduction	*73*	*72*	*75*	*71*	*67*
Acid soaking	1.00 ± 0.00^c	0.41 ± 0.01^c	0.32 ± 0.01^c	0.85 ± 0.02^c	0.97 ± 0.02^c
% Reduction	*59*	*61*	*66*	*62*	*59*
Alkali soaking	1.60 ± 0.01^b	0.51 ± 0.01^b	0.38 ± 0.01^b	1.58 ± 0.02^b	1.38 ± 0.01^b
% Reduction	*34*	*48*	*61*	*29*	*39*
MRL[a]	2.00	0.7	0.4	2.0	2.0

Means having similar letters in a column are statistically non–significant at $p \leq 0.05$, Mean ± S.E. (n = 2). SP, Spinach powder.
% Reduction = Rate of pesticides' reduction in treated forms of spinach.
[a] MRL – Maximum residue levels (Codex Alimentarius Commission, 2011).

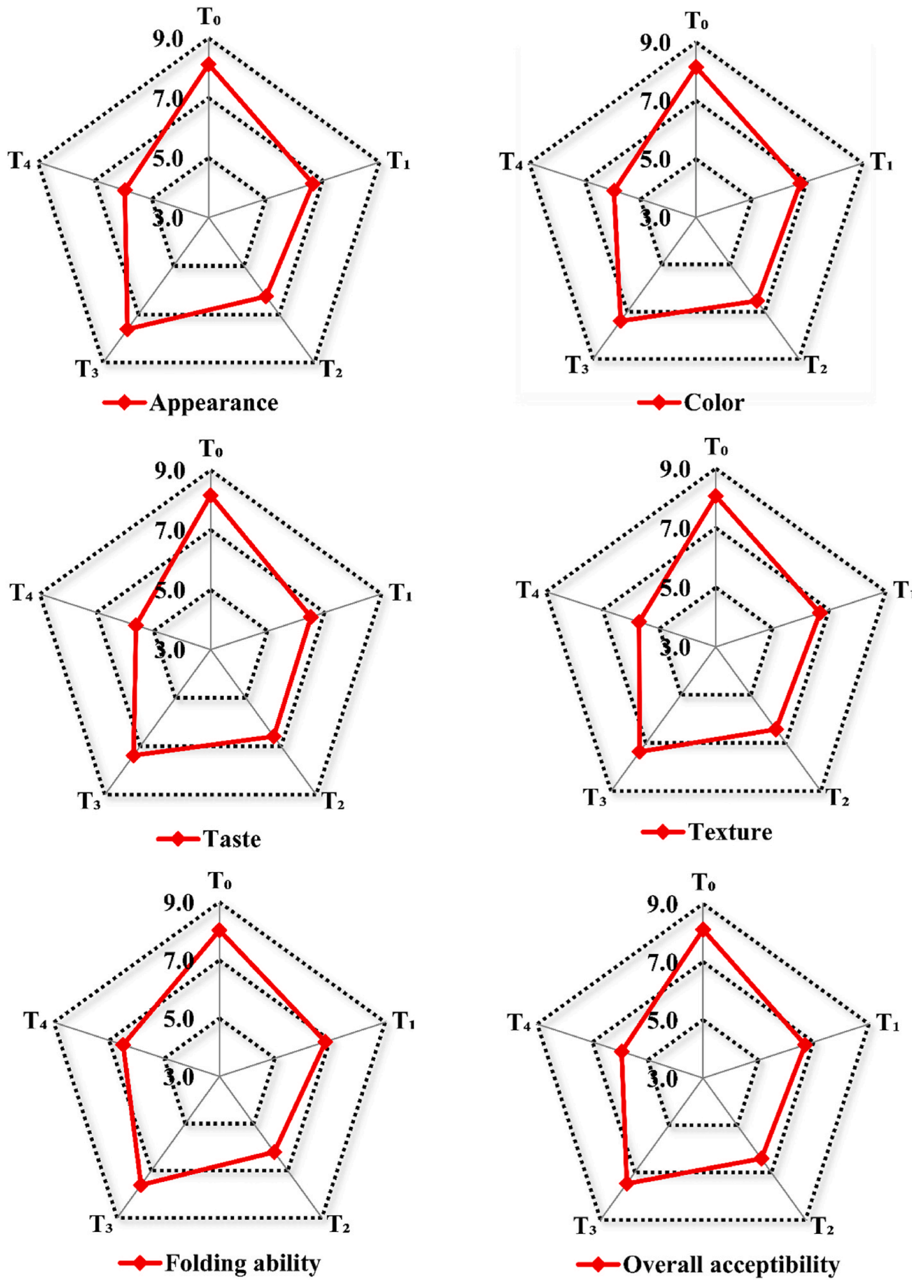

Fig. 2. Sensory qualities of microwave processed spinach powder supplemented unleavened flatbreads
T0 = Control (100% wheat flour unleavened flatbread without microwave treated spinach powder), T1 = 2.5% microwave treated spinach powder (SPmw), T2 = 5% SPmw, T3 = 7.5% SPmw, T4 = 10% SPmw.

control i.e., 8.15 and 8.15 followed by T_3 i.e., 7.4 and 7.37, respectively. Comparatively, T_4 (10% supplementation level) anticipated the least sensory acceptability for color, taste, texture and overall acceptability in microwaved processed chapatis. Addition of dehydrated SP at a rate of 2.5 to 10% in supplemented chapatis yielded sensory scores in a ranged between 5.6 to 7.6 (Fig. 2). Comparatively, low sensory scoring and reduced acceptability of processed chapatis beyond 7.5% supplementation level could be attributed to the presence of astringent substances such as isothiocyantes and tannins in spinach. Value addition of vegetables powders in development of baked goods is considered as a viable approach to mitigate micronutrient deficiencies among vulnerable population groups. Earlier studies have validated the use of nutrient rich dehydrated spinach powder up to 15% in development of biscuits wherein, the researchers revealed the best sensorial acceptability of value added biscuits with 5% spinach powder [34]. A recent study by Mahmudah et al. [54] and an earlier one by Nabila [55] also reported the better sensory acceptability of brown rice and green spinach powder based composite cookies and donuts wherein spinach powder supplementation at a rate of less than10% yielded an overall good sensory score for color and texture.

4. Conclusions

Spinach is suggested as a promising source of health promoting dietary components such as Fe, Zn, Ca, proteins, and dietary fibers exhibiting nutritional and health ameliorative activities. Contrarily, spinach is also supposed to uphold substantial concentrations of anti-nutrients and other hazardous substances like pesticides residues which may affect micronutrients bioavailability and anticipate several health malignancies. Processing of leafy green vegetables using domestic cooking techniques are considered as cost effective and reliable approaches to produce safer foods with better quality. Outcomes of our study advocate microwave heating, blanching and soaking to anticipate plausible reduction in levels of intrinsic and extrinsic toxicants in spinach powders. Our results strongly support application of microwave heating (i.e., 1.1 kW, 2 min) as a promising thermal technique to reduce antinutrients and pesticides residues to a safer level and improve nutritional potential of the spinach. Further, our findings suggest spinach powder utilization as a cost effective ingredient in improving the nutritional, technofunctional and thickening properties of savory foods such as traditional gravies, soups and curries. Our results also spotlight the future prospects of microwave heat processed spinach powder as a novel and a safer food-based ingredient in decreasing the ever-increasing burden of micronutrients deficiencies-, and in preventing health maladies such as obesity, diabetes and cancer.

Funding

The publication of this article was funded by the Open Access Fund of Leibniz Universität Hannover.

CRediT authorship contribution statement

Muhammad Waseem: Writing – original draft, Visualization, Software, Investigation, Formal analysis. **Saeed Akhtar:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Conceptualization. **Tahir Mehmood:** Software. **Muhammad Qamar:** Writing – original draft. **Wisha Saeed:** Writing – original draft, Formal analysis. **Muhammad Younis:** Formal analysis. **Saima Perveen:** Investigation. **Tariq Ismail:** Writing – original draft, Validation, Supervision, Methodology, Data curation, Conceptualization. **Tuba Esatbeyoglu:** Writing – review & editing, Validation, Conceptualization, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Authors are very grateful to the Chairman, Pakistan Agriculture Research Council (PARC), Agricultural Linkages Program (ALP) scheme.

References

[1] M. Waseem, S. Akhtar, M.F. Manzoor, A.A. Mirani, Z. Ali, T. Ismail, N. Ahmad, E. Karrar, Nutritional characterization and food value addition properties of dehydrated spinach powder, Food Sci. Nutr. 9 (2021) 1213–1221, https://doi.org/10.1002/fsn3.2110.

[2] S. Jyoti, V. Sangwan, Nutritional composition and sensory characteristics of Burfi supplemented with spinach leaves powder, Pharma Innov. 11 (2022) 766–770, https://doi.org/10.22271/tpi.

[3] V. Sangwan, S. Jood, Sensory and nutritional analysis of spinach powder fortified biscuits, Curr. Appl. Sci. Technol. 41 (2022) 10–16, https://doi.org/10.9734/cjast/2022/v41i831681.

[4] V. Vaštakaitė-Kairienė, A. Brazaitytė, J. Miliauskienė, E.S. Runkle, Red to blue light ratio and iron nutrition influence growth, metabolic response, and mineral nutrients of spinach grown indoors, Sustainability 14 (2022) 12564, https://doi.org/10.3390/su141912564.

[5] T. Sarkar, M. Salauddin, S. Roy, R. Chakraborty, M. Rebezov, M.A. Shariati, M. Thiruvengadam, K.R.R. Rengasamy, Underutilized green leafy vegetables: frontier in fortified food development and nutrition, Crit. Rev. Food Sci. Nutr. (2022) 1–55, https://doi.org/10.1080/10408398.2022.2095555.

[6] C.S. Lasya, Spinach and its health benefits: a review, Pharma Innov. 8 (2022) 1232–1239, https://doi.org/10.22271/tpi.

[7] A.A. Kureshi, S. Ghoshal, S. Adsare, G. Saste, A. Mirgal, A. Girme, L. Hingorani, Distinct morphological and analytical features of spinacia oleracea differentiating from conventional spinach plants, ACS Food Sci. Technol. 3 (2023) 273–282, https://doi.org/10.1021/acsfoodscitech.2c00300.

[8] M. Czarnowska-Kujawska, M. Starowicz, V. Barišić, W. Kujawski, Health-promoting nutrients and potential bioaccessibility of breads enriched with fresh kale and spinach, Foods 11 (2022) 3414, https://doi.org/10.3390/foods11213414.

[9] A. Sapozhnikov, L. Rozhdestvenskaya, A. Kopylova, Quality evaluation of bakery products enriched with spinach, in: IOP Conference Series: Earth and Environmental Science, IOP Publishing, 2019.

[10] M. Waseem, S. Akhtar, M. Qamar, W. Saeed, T. Ismail, T. Esatbeyoglu, Effect of thermal and non-thermal processing on nutritional, functional, safety characteristics and sensory quality of white cabbage powder, Foods 11 (2022) 3802, https://doi.org/10.3390/foods11233802.

[11] H. Alshemmari, A.E. Al-Shareedah, S. Rajagopalan, L.A. Talebi, M. Hajeyah, Pesticides driven pollution in Kuwait: the first evidence of environmental exposure to pesticides in soils and human health risk assessment, Chemosphere 273 (2021) 129688, https://doi.org/10.1016/j.chemosphere.2021.129688.

[12] V. De Luca, L. Mandrich, G. Manco, Development of a qualitative test to detect the presence of organophosphate pesticides on fruits and vegetables, Life 13 (2023) 490, https://doi.org/10.3390/life13020490.

[13] R.M. Amir, M.A. Randhawa, M. Nadeem, A. Ahmed, A. Ahmad, M.R. Khan, M. A. Khan, R. Kausar, Assessing and reporting household chemicals as a novel tool to mitigate pesticide residues in spinach (Spinacia oleracea), Sci. Rep. 9 (2019) 1125, https://doi.org/10.1038/s41598-018-37936-2.

[14] C.A. Commission, International Food Standards, 2011. https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/commodities-detail/en/?c_id=313. (Accessed 10 February 2023).

[15] R. Calderon, J. García-Hernández, P. Palma, J. Leyva-Morales, M. Zambrano-Soria, P. Bastidas-Bastidas, M. Godoy, Assessment of pesticide residues in vegetables commonly consumed in Chile and Mexico: potential impacts for public health, J. Food Compos. Anal. 108 (2022) 104420, https://doi.org/10.1016/j.jfca.2022.104420.

[16] M. Ali, M.F. Manzoor, G. Goksen, R.M. Aadil, X.-A. Zeng, M.W. Iqbal, J.M. Lorenzo, High-intensity ultrasonication impact on the chlorothalonil fungicide and its reduction pathway in spinach juice, Ultrason. Sonochem. 94 (2023) 106303, https://doi.org/10.1016/j.ultsonch.2023.106303.

[17] S.A. Mir, B. Dar, M.M. Mir, S.A. Sofi, M.A. Shah, T. Sidiq, K.V. Sunooj, A. M. Hamdani, A.M. Khaneghah, Current strategies for the reduction of pesticide residues in food products, J. Food Compos. Anal. 106 (2022) 104274, https://doi.org/10.1016/j.jfca.2021.104274.

[18] M. Samtiya, R.E. Aluko, T. Dhewa, Plant food anti-nutritional factors and their reduction strategies: an overview, Food Prod. Process. Nutr. 2 (2020) 1–14, https://doi.org/10.1186/s43014-020-0020-5.

[19] A.-A. Zhang, P.P. Sutar, Q. Bian, X.-M. Fang, J.-B. Ni, H.-W. Xiao, Pesticide residue elimination for fruits and vegetables: the mechanisms, applications, and future trends of thermal and non-thermal technologies, J. Future Foods 2 (2022) 223–240, https://doi.org/10.1016/j.jfutfo.2022.06.004.

[20] K. Amin, S. Akhtar, T. Ismail, Nutritional and organoleptic evaluation of functional bread prepared from raw and processed defatted mango kernel flour, J. Food Process. Preserv. 42 (2018) e13570, https://doi.org/10.1111/jfpp.13570.

[21] B. Chaudhari, N. Kamble, S. Waychal, S. Bansode, Standardization and estimation of production cost of paneer incorporated with spinach powder, Pharma Innov. 12 (2022) 71–76, https://doi.org/10.22271/tpi.2022.v11.i12a.17639.

[22] M. Shafi, W.N. Baba, F.A. Masoodi, R. Bazaz, Wheat-water chestnut flour blends: effect of baking on antioxidant properties of cookies, J. Food Sci. Technol. 53 (2016) 4278–4288, https://doi.org/10.1007/s13197-016-2423-5.

[23] L. Yu, H.S. Ramaswamy, J. Boye, Protein rich extruded products prepared from soy protein isolate-corn flour blends, LWT–Food Sci. Technol. 50 (2013) 279–289, https://doi.org/10.1016/j.lwt.2012.05.012.

[24] P. Sharma, H.S. Gujral, C.M. Rosell, Effects of roasting on barley β-glucan, thermal, textural and pasting properties, J. Cereal. Sci. 53 (2011) 25–30, https://doi.org/10.1016/j.jcs.2010.08.005.

[25] (AACC), A.A.o.C.C, in: Approved Methods of the American Association of Cereal Chemists, tenth ed., The Association, Saint Paul, MN, USA, 2000.

[26] A. Gani, S.M. Wani, F. Masoodi, R. Salim, Characterization of rice starches extracted from Indian cultivars, Food Sci. Technol. Int. 19 (2013) 143–152, https://doi.org/10.1177/1082013212442189.

[27] N. de Almeida Costa, L.R. Silveira, E. de Paula Amaral, G.C. Pereira, D. de Almeida Paula, É.N.R. Vieira, E.M.F. Martins, P.C. Stringheta, B.R.d.C.L. Júnior, A. M. Ramos, Use of maltodextrin, sweet potato flour, pectin and gelatin as wall material for microencapsulating Lactiplantibacillus plantarum by spray drying: thermal resistance, in vitro release behavior, storage stability and physicochemical properties, Food Res. Int. 164 (2023) 112367, https://doi.org/10.1016/j.foodres.2022.112367.

[28] J. Latimer, in: Official Methods of Analysis, nineteenth ed., Association of Official Analytical Chemists, Gaithersburg, MD, USA, 2019.

[29] G. Onwuka, Soaking, boiling and antinutritional factors in pigeon peas (Cajanus cajan) and cowpeas (Vigna unguiculata), J. Food Process. Preserv. 30 (2006) 616–630, https://doi.org/10.1111/j.1745-4549.2006.00092.x.

[30] W. Haug, H.J. Lantzsch, Sensitive method for the rapid determination of phytate in cereals and cereal products, J. Sci. Food Agric. 34 (1983) 1423–1426, https://doi.org/10.1002/jsfa.2740341217.

[31] M. El-Saeid, M. Selim, Effect of food processing on reduction of pesticide residues in vegetables, J. Appl. Life Sci. Int. 8 (2016) 1–6, https://doi.org/10.9734/JALSI/2016/26801.

[32] Abdullah, M.A. Randhawa, S. Akhtar, A. Asghar, M. Sohaib, R.M. Aadil, M. A. Jahangir, Assessment of different washing treatments to mitigate imidacloprid and acetamaprid residues in spinach, J. Sci. Food Agric. 96 (2016) 3749–3754, https://doi.org/10.1002/jsfa.7563.

[33] M. Suriya, G. Baranwal, M. Bashir, C.K. Reddy, S. Haripriya, Influence of blanching and drying methods on molecular structure and functional properties of elephant foot yam (Amorphophallus paeoniifolius) flour, LWT–Food Sci. Technol. 68 (2016) 235–243, https://doi.org/10.1016/j.lwt.2015.11.060.

[34] N.R. Galla, P.R. Pamidighantam, B. Karakala, M.R. Gurusiddaiah, S. Akula, Nutritional, textural and sensory quality of biscuits supplemented with spinach (Spinacia oleracea L.), Int. J. Gastron. Food Sci. 7 (2017) 20–26, https://doi.org/10.1016/j.ijgfs.2016.12.003.

[35] M. Khan, C. Mahesh, A.D. Semwal, G. Sharma, Effect of spinach powder on physico-chemical, rheological, nutritional and sensory characteristics of chapati premixes, J. Food Sci. Tech. Mys. 52 (2015) 2359–2365, https://doi.org/10.1007/s13197-013-1198-1.

[36] N.N. Tafu, V.A. Jideani, Proximate, elemental, and functional properties of novel solid dispersions of Moringa oleifera leaf powder, Molecules 27 (2022) 4935, https://doi.org/10.3390/molecules27154935.

[37] A. Chib, A. Bhat, J.D. Bandral, M. Trilokia, Effect of thermal processing on nutritional and Anti nutritional factors of amaranthus (*Amaranthus viridis* Linn.) Leaves, Pharma Innov. 11 (2022) 385–389.

[38] N.K. Huynh, D.H. Nguyen, H.V. Nguyen, Effects of processing on oxalate contents in plant foods: a review, J. Food Compos. Anal. 112 (2022) 104685, https://doi.org/10.1016/j.jfca.2022.104685.

[39] Z. Wang, A. Ando, A. Takeuchi, H. Ueda, Effects of cooking conditions on the relationships among oxalate, nitrate, and lutein in spinach, Food Sci. Technol. Res. 24 (2018) 421–425, https://doi.org/10.3136/fstr.24.421.

[40] M.S. Akhtar, B. Israr, N. Bhatty, A. Ali, Effect of cooking on soluble and insoluble oxalate contents in selected Pakistani vegetables and beans, Int. J. Food Prop. 14 (2011) 241–249, https://doi.org/10.1080/10942910903326056.

[41] Y. Shimada, The eeffect of soaking on the soluble oxalic acid content of spinach, Chugokugakuen J 13 (2014) 27–31. https://cur-ren.repo.nii.ac.jp/records/186.

[42] A. Kumoro, C. Budiyati, D. Retnowati, Calcium oxalate reduction during soaking of giant taro (*Alocasia macrorrhiza* (L.) Schott) corm chips in sodium bicarbonate solution, Int. Food Res. J. 21 (2014) 1583–1588.

[43] B. Kala, V. Mohan, Effect of microwave treatment on the antinutritional factors of two accessions of velvet bean, *Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) Bak. ex Burck, Int. Food Res. J. 19 (2012).

[44] S.I. Rafiq, S. Singh, D.C. Saxena, Physical, physicochemical and anti-nutritional properties of Horse Chestnut (*Aesculus indica*) seed, J. Food Meas. Char. 10 (2016) 302–310, https://doi.org/10.1007/s11694-016-9307-2.

[45] R. Vashishth, A. Semwal, M. Naika, G. Sharma, R. Kumar, Influence of cooking methods on antinutritional factors, oligosaccharides and protein quality of underutilized legume *Macrotyloma uniflorum*, Food Res. Int. 143 (2021) 110299, https://doi.org/10.1016/j.foodres.2021.110299.

[46] A. Bonnechère, V. Hanot, R. Jolie, M. Hendrickx, C. Bragard, T. Bedoret, J. Van Loco, Effect of household and industrial processing on levels of five pesticide residues and two degradation products in spinach, Food Control 25 (2012) 397–406, https://doi.org/10.1016/j.foodcont.2011.11.010.

[47] L. Zhao, F. Liu, J. Ge, L. Ma, L. Wu, X. Xue, Changes in eleven pesticide residues in jujube (*Ziziphus jujuba* Mill.) during drying processing, Dry. Technol. 36 (2018) 965–972, https://doi.org/10.1080/07373937.2017.1367306.

[48] Y. Wu, Q. An, D. Li, J. Wu, C. Pan, Comparison of different home/commercial washing strategies for ten typical pesticide residue removal effects in kumquat, spinach and cucumber, Int. J. Environ. Res. Publ. Health 16 (2019) 472, https://doi.org/10.3390/ijerph16030472.

[49] M. Park, H. Kim, M. Kim, M.-h. Im, Reduction in residual cyantraniliprole levels in spinach after various washing and blanching methods, Front. Nutr. 9 (2022) 948671, https://doi.org/10.3389/fnut.2022.948671.

[50] S.W. Chung, How effective are common household preparations on removing pesticide residues from fruit and vegetables? A review, J. Sci. Food Agric. 98 (2018) 2857–2870, https://doi.org/10.1002/jsfa.8821.

[51] T.T. Nguyen, C. Rosello, R. Bélanger, C. Ratti, Fate of residual pesticides in fruit and vegetable waste (FVW) processing, Foods 9 (2020) 1468, https://doi.org/10.3390/foods9101468.

[52] Z. Siddique, A.U. Malik, M.R. Asi, M. Inam-Ur-Raheem, M. Abdullah, Impact of sonolytic ozonation (O_3/US) on degradation of pesticide residues in fresh vegetables and fruits: case study of Faisalabad, Pakistan, Ultrason. Sonochem. 79 (2021) 105799, https://doi.org/10.1016/j.ultsonch.2021.105799.

[53] H.-W. Xiao, Z. Pan, L.-Z. Deng, H.M. El-Mashad, X.-H. Yang, A.S. Mujumdar, Z.-J. Gao, Q. Zhang, Recent developments and trends in thermal blanching–A comprehensive review, Inf. Process. Agric. 4 (2017) 101–127, https://doi.org/10.1016/j.inpa.2017.02.001.

[54] N.A. Mahmudah, R.N. Mauizza, H. Nabawiyah, A.Y. Damayanti, Nutritional value and sensory properties of Brown rice flour cookies with green spinach *(Amaranthus tricolor* L.) incorporation as gluten-free food alternative, J. Teknologi Pangan dan Hasil Pertanian 18 (2023) 33–40, https://doi.org/10.26623/jtphp.v18i1.6110.

[55] F. Nabila, Effect of substitution of spinach flour (*Amaranthus* sp) to iron (fe) content and acceptability of donuts, J. Nutraceuticals Herbal Medicine 2 (2019) 1–11, https://doi.org/10.23917/jnhm.v2i1.6706.