J. of Food and Dairy Sci., Mansoura Univ., Vol. 15 (10):133 - 142, 2024

Journal of Food and Dairy Sciences

Journal homepage & Available online at: www.jfds.journals.ekb.eg

Enhancing Protein Digestibility, Bioaccessibility, and Nutrient Retention in Faba Beans Using Nano-Zinc Oxide and Thermal Treatments

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ABSTRACT

This study examines the impact of thermal treatments (steaming and autoclaving) and nano-zinc oxide (Nano-ZnO) foliar applications on the nutritional properties of Faba bean (*Vicia faba L.*) variety Sakha 1. Changes in chemical composition, mineral contents, particle size distribution, color properties, protein digestibility, bioaccessibility, antioxidant activity, and phenolic compound content. Thermal processing treatments especially autoclaving, significantly enhanced protein, dietary fiber, and ash content, also showing the highest protein digestibility and bioaccessibility for Faba seeds. While Nano-ZnO applications further increase protein levels, with steamed beans which treated with 2 $g \cdot L^{-1}$ Nano-ZnO showing the most significant improvements. These combined treatments also reduce phytic acid levels and boost antioxidant activity, particularly in steamed Faba seeds samples. Thermal treatments generally lower mineral content in compare with the other samples, Nano-ZnO applications, especially at 2 $g \cdot L^{-1}$, retain higher mineral levels compared to untreated controls. FTIR analysis indicates structural changes in proteins due to thermal treatments, and color analysis shows significant variations in lightness and color components. In vitro protein digestibility significantly improves with thermal treatments, reaching 80.83% in autoclaved Faba samples. Protein bioaccessibility also increases, with the highest value of 33.71% observed in autoclaved beans treated with Nano-ZnO.

Keywords: Faba bean, Steaming, Autoclaving, Digestibility, Nano-zinc oxide.

INTRODUCTION

Legumes, which contain a significant amount of protein, are essential in the diets of those living in low-income areas. After cereals, they are considered the second most important staple food. Legumes also include a high concentration of dietary fibers, complex carbohydrates, minerals, and vitamins, all of which play essential roles in energy production (Medhe *et al*., 2019). Fava beans (*Vicia faba L.*) are one of the oldest and most esteemed crops in human nutrition, holding the fifth position in global legume production. Their significance in dietary practices stems from their rich protein and bioactive compound profile, which showcases their capacity to support human health and prevent diseases (Ayala-Rodríguez *et al*., 2022).

In comparison to cereals, dry fava bean seeds have a high lysine concentration of 19.8 g / kg of dry matter and low contents of methionine, cysteine, and tryptophan, which are 2.6, 3.7, and 2.7 g/ kg of dry matter, respectively (Vioque *et al*., 2012). Hence, investigating fava bean proteins is intriguing due to their nutritional and physiological significance. Furthermore, the protein extract derived from the fava bean can be utilized in powdered form across various food items (Alpizar-Reyes *et al*., 2018).

Legumes are typically not eaten raw; therefore, various thermal processing techniques enhance their sensory appeal and sometimes improve their nutritional value. Thermal processing is crucial for extending food shelf life and enhancing sensory and nutritional qualities (Ahmed and Eun, 2018; Duan *et al*., 2021) because it gelatinizes the starch, modifies the texture, and improves its flavor; in this way, legumes are made palatable (Rahate *et al*., 2021). Studies have shown changes in Faba bean phytochemicals after thermal treatments, such as increased phytic acid and decreased tannins (Luo and Xie, 2013). Furthermore, moderate heat increases protein availability in most legumes and weakens intercellular binding material, making it possible that pressure from a fork or teeth could cause intact starch-filled cells to separate. Heat also inactivates or reduces the concentration of non-nutritional compounds such as protease and lectin inhibitors. Common cooking methods for dry legumes include boiling, pressure boiling, and steaming, with soaking as a preliminary step to soften the texture and reduce cooking time. Autoclaving processing also enhances food quality, preserving flavor, color, and biologically active components (Rahate *et al*., 2021).

Nanotechnology increases crop yields by improving valuable inputs and reducing waste (Shang *et al*., 2019). Nanomaterials, with their large surface area, are effective carriers for intelligent nutrient delivery systems, boosting plant growth and resilience while increasing yield (El-Henawy *et al*., 2018; Merghany *et al*., 2019). Nanofertilizers, especially zinc oxide nanoparticles (nano-ZnO), provide controlled nutrient release and minimize soil toxicity (Kah *et al*., 2018; Subramanian *et al*., 2015). Nano-ZnO is widely used and recognized as safe by the US FDA (El-Sharkawy *et al*., 2021). However, the impact of thermal processing on the phytochemical content, antioxidant activity, protein digestibility, and bioaccessibility of Faba beans treated with nano-ZnO remains understudied.

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DOI: 10.21608/jfds.2024.318467.1166

This study explores the impact of thermal treatments (steaming, autoclaving) on the chemical and nutritional components of the Faba bean variety Sakha 1 with foliar applications of nano-ZnO. It also aims to provide scientific information on the digestibility and bioaccessibility of protein flours of fava bean seeds.

MATERIALS AND METHODS

Materials

The Faba bean (*Vicia faba L.*) variety (Sakha 1) was sourced from the Sakha Agricultural Research Station Farm, located in Kafr El-Sheikh Governorate, Egypt, during the winter season of 2022. The radicals ABTS and DPPH, were sourced from Sigma Aldrich Co., Ltd., located in St. Louis, MO, USA. For the in vitro gastrointestinal system simulation, we acquired pepsin, pancreatin, bile salts, and Membra-Cel MD34 dialysis bags from Sigma-Aldrich Chemie GmbH & Co. KG (Steinheim, Germany).

Preparing Faba beans zinc oxide nanoparticles.

The experiments were conducted in split block design with three replications, the main plots were assigned as different concentrations of a foliar application of nano-zinc oxide (Nano-ZnO) at 1 and 2 $g \cdot L^{-1}$ under the same production system and one soil type following the methodology outlined by El-Sharkawy *et al*. (2021). Based on the Nano-ZnO concentration, the samples were labeled as Control (without any Nano-ZnO), Nano-ZnO1(treated with 1 $g \cdot L^{-1}$), and Nano-ZnO2 (treated with 2 $g \cdot L^{-1}$)

Autoclaving and Steaming process for Faba bean.

To obtain the autoclaved fava beans, the seeds of all treatment (Control, Nano-ZnO1, and Nano-ZnO2) were soaked in distilled water at a ratio of 1:5 w/v and refrigerated for 12 hours at 4°C. The seeds were washed three times using distilled water and left to dry. They were immediately transferred to 1-liter beakers, and 100 mL of distilled water was added before autoclaving (Yamato Scientific SM300 America Inc.) at 120°C for 10 minutes (Khalil, 2001). The seeds were dried at 50°C for 48 hours, with continuous stirring to ensure even drying

To obtain the steamed fava beans, A liter of distilled water was poured into a pot with a tray and a lid to steam Faba beans. The seeds of all treatment (Control, Nano-ZnO1, and Nano-ZnO2) were placed on the tray when the water boiled, and the lid was quickly closed. The seeds were dried at 50°C for 48 hours, with continuous stirring to ensure even drying. Autoclaved and steamed dried seeds are crushed and ground (KA® A 11 analytical mill). The flour is filtered through an 850 µm mesh screen and then lyophilized to ensure homogeneity (Siah *et al*., 2014).

Proximate composition.

Proximal chemical analyses for all Faba seeds were conducted following the procedures outlined by Aoac (2005). The analyses included protein (Method 955.04, N x 6.25), total dietary fiber (Method 923.03), ash content (Method 923.03), total lipids (Method 920.39), and carbohydrates, which were calculated by difference.

Minerals Content.

Samples from each treatment underwent digestion using a mixture of H2SO4 and H2O2, following the method described by Peterburgski (1968). Total P and N contents were measured using the technique outlined by Page Blume (1985). The potassium (K) content was measured using a flame photometer (Cottenie *et al*., 1982), while the zinc content was assessed with an atomic absorption spectrophotometer, following the method described by Chapman and Pratt (1962).

Particle size and charge analysis

Particle size determination was conducted using dynamic light scattering with the assistance of a Zetasizer instrument (Malvern Instruments, Malvern, U.K.). The samples were hydrated in water at a 1:10 ratio with distilled water.

Extraction of phenolic compounds.

The method for extracting polyphenols was modified from the one described by Saleh *et al*. (2024). Ten grams of sample were dissolved in 100 mL of 75% (v/v) aqueous ethanol. The solution was then sonicated in a cooled ultrasonic bath to maintain the temperature below 4 °C for 30 minutes, followed by centrifugation at 4 °C and 2700g for 10 minutes. The supernatants were carefully collected. The solvent was then removed by rotating an evaporator at 60 °C. Rotating an evaporator at 60°C was used to extract the solvent. Finally, the Faba bean flour extracts were stored at -20 °C in a brown vial until further investigation.

Determination of total polyphenols compounds.

Total polyphenol content determination was performed using the method described by Peanparkdee *et al*. (2020) with some modifications. A 2.5 mL of Folin-Ciocalteu reagent (0.2 N) was added to 500 µL of the extracted sample. The mixture was left to stand for 5 minutes. Following this, 2 mL of a 7.5% sodium carbonate (Na2CO3) solution was added, and the samples were allowed to stand for 120 minutes in the dark. The absorbance was measured at 765 nm (UV2500UV–Vis, Shimadzu Co., Japan)

Antioxidant activity

DPPH assay

A DPPH (2,2-diphenyl-1-picrylhydrazyl) analysis was conducted, followed by Peanparkdee *et al*. (2020). The radical scavenging capacity was assessed using the DPPH method. A 0.5 mL sample was combined with 5 mL of a 0.1 mM DPPH solution and allowed to stand at room temperature for 30 minutes before the absorbance was measured at 517 nm.

ABTS assay

The ABTS assay was conducted following the methodology described by Prasedya *et al*. (2021).

After 16 hours in the dark, a reaction between 5 mL of a 7 mM ABTS aqueous solution and 88 mL of a 140 mM K2S2O8 solution formed an ABTS•+ solution. A sample of 20 µL was then mixed with 230 µL of the ABTS•+ solution and incubated in the dark for two hours. The absorbance of this mixture was subsequently measured at 734 nm (Thermo Scientific Evolution 300 spectrophotometer).

Color properties

The color of each flour sample was measured with a colorimeter (Minolta CR-4, Japan). The parameters L*, a*, and b* indicate lightness/darkness, redness/greenness, and yellowness/blueness, respectively. The overall color difference (ΔE) is calculated using the following equation:

$$\Delta E = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{1/2}$$

The values of Croma and hue angle were quantified as follows:

$$\text{Hue angle} = [\tan^{-1} (b/a)]$$

$$\text{Chroma} = [(a^2 + b^2)]^{1/2}$$

Functional properties of faba flour.

FTIR of fava bean flour.

The sample flours were analyzed using an attenuated total reflectance (ATR) Fourier transform infrared (FT-IR) spectrometer (Nicolet Avatar 36) to get their infrared spectra in the range of 500 to 4000 cm-1.

Protein concentrates.

The defatting of flour was conducted using the methodology described by Serrano-Sandoval *et al*. (2019). The samples were treated with a hexane solution (1:4 w/v) and stirred continuously at 250 rpm for four hours. The suspension underwent vacuum filtration using a glass microfibre filter and was subsequently dried for 24 hours. Subsequently, 20 g of each sample was combined with 200 mL of distilled water, and the pH was adjusted to 8.5 using 1 M NaOH, followed by stirring for 2 hours. The samples were centrifuged for 20 minutes at 10,000 g and 4 °C. The supernatant was collected, and the pH was adjusted to 4.5 using 1M HCl. Subsequently, the samples were stirred for two hours and centrifuged once more. The final pellet from this last step was freeze-dried and preserved for subsequent analysis.

In vitro protein digestibility (IVPD).

The standardized international consensus protocol INFOGEST, as outlined by Brodkorb *et al*. (2019), with slight modifications. Simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) have been prepared as solutions that replicate the physiological fluids that are involved in human digestion. To calculate IVPD, 1 mL of digested samples (intestinal phase) was added to a 1.5 mL Eppendorf™ microtube and centrifuged at 15,000 rpm for 10 minutes at room temperature. Proteins in the supernatant were soluble, while the residue was insoluble and perhaps non-absorbable. Digestibility was calculated. The formula to calculate the IVPD is as follows (Li *et al*., 2017)

$$IVPD\ (\%) = 100 \times P_{t0} - P_{t1} / Pt0$$

The protein content of the sample without treatment before digestion is indicated as Pt0, while the protein content of the sample residue after intestinal digestion is indicated as Pt1.

In vitro protein bioaccessibility (IVPB*).*

The static dialysis method used by Managa *et al*. (2021) was used to study IVPB with a cellulose membrane. Digested intestinal phase samples (8 mL) were placed in a cellulose dialysis membrane (D9652, Sigma-Aldrich). Then, each dialysis tube was submerged in 40 mL of SIF. The mixture was incubated in a water bath at 37°C for 120 minutes with gentle manual stirring every 15 minutes. This formula calculates IVPB (Liu *et al*., 2021).

$$IVPB\ (\%) = P_{td} / P_{ti} \times 100$$

P_{td} is the dialyzed protein of the digested samples; P_{ti} is the protein content in the initial undigested sample.

Statistical analysis.

A one-way ANOVA was used to determine the mean values and their respective standard deviations. Duncan's multiple-range tests determined the significance at the $p<0.05$ level. SPS software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis (Alshehri *et al*., 2024).

RESULTS AND DISCUSSION

Chemical composition of raw, Steamed, and Autoclaved Faba bean.

Table (1) presents the chemical composition of raw, steamed, and autoclaved Faba bean seeds under different treatments. Protein content is an essential component of nutritional analysis, indicating the amount of essential amino acids available in food. This study examined the protein content of raw, steamed, and autoclaved Faba bean seeds under different treatments, including a control group and groups treated with Nano-ZnO1 and Nano-ZnO2.

For the control group, the protein content in raw faba bean seeds was 31.67%. The results are consistent with (Martínez-Velasco *et al*., 2018; Rahate *et al*., 2021; Warsame *et al*., 2018), who reported that Vicia Faba protein content ranges from 26 to 36 g/100g

Steaming increased the protein content to 34.81%, suggesting that the steaming process might concentrate the protein by reducing the moisture content or making proteins more accessible. Autoclaving also increased protein content to 33.39%, indicating that high-pressure cooking can enhance protein concentration, possibly through mechanisms similar to steaming. According to other investigators, the increased protein content in cooked samples compared to raw samples is due to heat processing enhancing the ability of proteins to be digested. This is achieved by destroying protease inhibitors and altering the protein structure through denaturation, as explained by Onwuka and Okala (2003). Corzo-Ríos *et al*. (2020) found similar results that cooked bean samples had a protein increase of 0.5-2.2% due to the loss of soluble solids during cooking, which increased the concentration of starch and protein.

Table 1. Effects of Steaming, Autoclaving, and Nano-Zinc Oxide Foliar Application on the Chemical Composition of Faba Bean.

Treatment		Protein	Dietary Fiber	Ash	Fat	Carbohydrates
Control	Raw	31.67± 0.41^{e}	5.31± 0.07^{c}	3.36± 0.08^{c}	1.71± 0.16^{a}	57.95±2.83^{a}
	Steamed	34.81 ± 0.15^{c}	5.62 ± 0.17^{a}	3.11 ± 0.14^{e}	1.42 ± 0.15^{b}	55.04±1.28^{b}
	Autoclaved	33.39± 0.19^{d}	5.47± 0.27^{b}	3.19± 0.18^{e}	1.25± 0.36^{b}	56.70±1.32^{a}
Nano-ZnO1	Raw	34.67± 0.26^{c}	5.27± 0.18^{c}	3.51± 0.17^{b}	1.73±0.16^{a}	54.82±1.14^{b}
	Steamed	35.47± 0.13^{b}	5.43± 0.12^{b}	3.27± 0.09^{d}	1.34± 0.18^{b}	54.49±1.27^{b}
	Autoclaved	36.26± 0.32^{a}	5.41± 0.10^{b}	3.31± 0.16^{c}	1.30± 0.27^{b}	53.72± 1.38^{c}
Nano-ZnO2	Raw	35.89± 0.19^{b}	5.43± 0.10^{b}	3.68± 0.07^{a}	1.78± 0.11^{a}	53.22± 1.53^{c}
	Steamed	36.72± 0.40^{a}	5.68± 0.12^{a}	3.32± 0.11^{c}	1.50± 0.26^{b}	52.78±1.66^{d}
	Autoclaved	36.31± 0.39^{a}	5.55± 0.29^{a}	3.37± 0.15^{c}	1.47± 0.17^{b}	53.30±1.78^{c}

Values are presented as means ± SD. Different lowercase letters within a column indicate significant differences at $p \leq 0.05$.

In the Nano-ZnO1 Faba sample, the raw Faba seeds had a higher protein content of 34.67%, indicating that the treatment with zinc oxide nanoparticles might enhance the protein content or its detectability. treated steamed seeds increased the protein content to 35.47%, showing an additive effect of the nanoparticles and the steaming process. Autoclaving process resulted in the highest protein content in this group at 36.26%. The combination of Nano-

ZnO1 treatment and autoclaving significantly enhances protein concentration.

The Nano-ZnO2 Faba sample demonstrated the highest raw protein content at 35.89%, indicating a strong effect of these nanoparticles on protein enhancement. Steaming process increased the protein content to 36.72%, the highest among all steamed samples, suggesting that Nano-ZnO2 might be more effective than Nano-ZnO1 in enhancing protein levels during the steaming process. Autoclaving also maintained a high protein content of 36.31%, similar to the steamed value, reinforcing the effectiveness of Nano-ZnO2 in preserving or enhancing protein content under different processing methods. A study conducted by Amin and Badawy (2017) found that the protein content of common bean plants increased when they were exposed to varying concentrations of Nano-ZnO (25, 50, 100, and 200 ppm). The protein content varies significantly across treatments, with a general trend of increases due to steaming and autoclaving. Nano-ZnO1 and Nano-ZnO2 notably enhance protein content, with Nano-ZnO2 showing a more pronounced effect. These findings suggest that processing methods and nanoparticle treatments can significantly influence the protein content of Faba bean seeds, potentially enhancing their nutritional value.

Dietary fiber content is a critical nutritional component, reflecting the indigestible parts of plant foods that aid digestion and have various health benefits. for the control group, the dietary fiber content in raw Faba bean seeds was 5.31%. Steaming increased the fiber content slightly to 5.62%, possibly due to the breakdown of cell walls, making the fiber more accessible. Autoclaving resulted in a dietary fiber content of 5.47%, a slight increase from the raw state, suggesting that the high-pressure cooking process may enhance fiber solubility or availability. The formation of tannin-protein complexes and resistant starch may lead to alterations in dietary fiber content during food preparation, potentially increasing the dietary fiber (Corzo-Ríos *et al*., 2020).

In the Nano-ZnO1 Faba beans sample, the raw seeds had a dietary fiber content of 5.27%, very close to the control group's raw value. Steaming these treated seeds resulted in a slight increase to 5.43%, indicating a minor effect of the nanoparticles combined with the steaming process. Autoclaving the Nano-ZnO1 treated seeds showed a dietary fiber content of 5.41%, similar to the steamed value, suggesting that autoclaving consistently impacts fiber content in the presence of Nano-ZnO1.

The Nano-ZnO2 Faba beans sample demonstrated slightly higher dietary fiber content in raw seeds at 5.43%, indicating a potential effect of the zinc oxide nanoparticles on fiber content. Steaming increased the fiber content to 5.68%, the highest among all steamed samples, suggesting an enhanced impact of Nano-ZnO2 on fiber availability. Autoclaving resulted in a dietary fiber content of 5.55%, maintaining the trend of higher fiber content than the control group and Nano-ZnO1 treatments. The dietary fiber content shows minor variations across treatments, with a general trend of slight increases due to steaming and autoclaving. Nano-ZnO2 appears to have a more pronounced effect on enhancing dietary fiber content than Nano-ZnO1, particularly in the steamed samples.

Similarly, Corzo-Ríos *et al*. (2022) observed a rise in the overall dietary fiber content of fava beans following the cooking process. Cooking increases the degradation of fiber constituents (lignin, cellulose, gums, hemicellulose, pectin, etc.), resulting in gelatinization and resistant starch formation. This process enhances the association of these chemicals with lipids and proteins. Furthermore, it causes significant qualitative and/or quantitative alterations that enhance dietary fiber (Sánchez-Arteaga *et al*., 2015).

The ash content in food chemistry refers to the total mineral content that remains after the complete combustion of organic matter in a food sample. For the control group, the ash content in raw Faba bean seeds was 3.36%. Steamed seeds slightly reduced the ash content to 3.11%, likely due to minerals leaching into the steaming water. Autoclaving further reduced the ash content to 3.19%, indicating that intense heat and pressure might cause some mineral loss or alteration. In the Nano-ZnO1 treated group, the raw seeds had an increased ash content of 3.51%, suggesting that zinc oxide nanoparticles enriched the mineral content. Steaming these treated seeds reduced the ash content to 3.27%, a trend similar to the control group but still higher due to the initial mineral enhancement from the nanoparticles. Autoclaved slightly reduced ash content to 3.31%, maintaining higher levels than the Control due to the nanoparticle treatment.

The Nano-ZnO2 Faba beans sample showed the highest ash content in raw seeds at 3.68%, indicating significant mineral enhancement compared to the control and Nano-ZnO1 treatments. Steaming reduced this to 3.32%, while autoclaving brought it down to 3.37%. Despite these reductions, the ash content remained higher than the control group, demonstrating the effectiveness of Nano-ZnO2 in increasing the mineral content of Faba bean seeds. Previous studies reported similar results when evaluating the effects of cooking methods on legumes (Kumar *et al*., 2022; Medhe *et al*., 2019). The percentage of fat present in raw flour was 1.69 %, which was not significantly different from that in Raw Nano-ZnO1 faba sample (1.73 %) and Raw Nano-ZnO2 (1.78 %). The Thermal processing significantly reduced the Fat percentage in the sample flour. According to Kumar *et al*. (2020), the reduction can be attributed to the breakdown and dispersion of lipids into the cooking water.

Effect of Thermal Treatments on the Mineral Content of Faba Bean.

Table (2) presents the mineral content of Faba bean under different treatments (raw, steamed, autoclaved). Both steaming and autoclaving in control samples decrease the mineral content (N, P, K, Zn) compared to raw Faba bean. The reduction is more pronounced in phosphorus content, especially in the autoclaved samples. Similar trends with decreased mineral content upon steaming and autoclaving in Nano-ZnO1 are observed. However, the decrease is less pronounced compared to the control samples.

Nano-ZnO2 Faba bean samples show the highest mineral content among all treated samples. The N content have highest amount in the Nano-ZnO2 Faba bean Samples and lowest in the control group. Similar to N and P, K content is highest in Nano-ZnO2 samples.

Steaming and autoclaving process in Nano-ZnO2 samples also reduce mineral content, but the samples still maintain higher mineral levels than the control and Nano-ZnO1 samples. Nano-ZnO treatment increases the mineral content significantly. Thermal treatments reduce zinc content, but the reduction is less pronounced in Nano-ZnO-

treated samples. According to Priyanka *et al*. (2019), nano-ZnO has the ability to improve the cation exchange capacity of roots, which in turn leads to an increase in the absorption of other essential nutrients, particularly zinc. The data indicates that thermal treatments (steaming and autoclaving) reduce the mineral content in Faba beans. However, foliar application of Nano-ZnO, particularly at 2 $g \cdot L^{-1}$ concentration, helps retain higher levels of minerals than the Control. This suggests that Nano-ZnO treatment can mitigate the loss of minerals due to thermal processing, making it a beneficial addition for maintaining the nutritional quality of Faba beans.

Table 2. Effects of Thermal Treatments, and Nano-Zinc Oxide Foliar Application on the mineral content of Faba bean seeds.

Treatment		N (%)	P (%)	K (%)	Zn (mg kg^{-1})
Control	Raw	0.62 ± 0.02^{e}	0.17 ± 0.03^{e}	0.30 ± 0.02^{e}	6.90 ±0.02^{e}
	Steamed	0.55 ± 0.03^{f}	0.14 ± 0.02^{f}	0.22 ± 0.03^{f}	6.67 ± 0.14^{f}
	Autoclaved	0.58 ± 0.03^{f}	0.11 ± 0.03^{g}	0.23 ± 0.02^{f}	6.59 ± 0.12^{f}
Nano-ZnO 1	Raw	0.97 ± 0.02^{c}	0.26 ± 0.02^{c}	0.55 ± 0.04^{c}	11.70 ± 0.07^{c}
	Steamed	0.93 ± 0.02^{d}	0.21 ± 0.02^{d}	0.51 ± 0.03^{d}	11.16 ± 0.15^{d}
	Autoclaved	0.96 ± 0.03^{c}	0.22 ± 0.02^{d}	0.50 ± 0.02^{d}	11.63 ± 0.16^{c}
Nano-ZnO 2	Raw	1.35 ± 0.03^{a}	0.37 ± 0.03^{a}	0.74 ± 0.03^{a}	15.81 ± 0.13^{a}
	Steamed	1.26 ± 0.02^{b}	0.31 ± 0.12^{b}	0.67 ± 0.03^{b}	15.47 ± 0.21^{b}
	Autoclaved	1.29 ± 0.03^{b}	0.32 ± 0.03^{b}	0.71 ± 0.02^{a}	15.78 ± 0.16^{a}

Values are presented as means ± SD. Different lowercase letters within a column indicate significant differences at $p \leq 0.05$.

Total Phenolic compounds, Phytic acid and antioxidant activity (DDPH and ABTS) of steaming and autoclaving of Faba bean

Table 3 shows the Total Phenolic compounds, Phytic acid and antioxidant activity for Faba bean after steaming, autoclaving, and nano-ZnO foliar application at 1 $g \cdot L^{-1}$ and 2 $g \cdot L^{-1}$ concentrations in Faba beans. The total phenolic component content in the Control, Nano-ZnO1, and Nano-ZnO2 raw samples were 169.6, 167.88, and 162.53 mg GAE/100g, respectively. Legume seeds contain different types and concentrations of phenolic compounds depending on their cultivation method and environmental conditions (Sánchez-Velázquez *et al*., 2021). The findings align with the Mayer Labba *et al*. (2021) study, which recorded a 1.4-5.0 mgGAE/g range for 15 fava bean seed cultivars.

Steaming and autoclaving process resulted in a significant decrease in TPC compared to the raw Faba beans samples in all samples. The findings are consistent with those of Siah *et al*. (2014), who observed that the reduction in phenolic contents of beans while cooking may be attributed to the degradation of phenolic compounds or the formation of new insoluble components with other organic substances through chemical changes.

Phytic acid is the most essential substance that causes phosphorus storage in legumes (Martineau-Côté *et al*., 2023). The raw Faba beans samples had phytic acid levels ranging from 8.58 to 9.69 mg/g dry weight, as shown in Table 3. This is within the range of phytic acid levels recorded for other cultivars, which is 1.12 to 12.81mg/g dry weight, as reported by Corzo-Ríos *et al*. (2022); Mayer Labba *et al*. (2021). The thermal treatment significantly decreased the levels of phytates in the control sample. The control sample exhibited 6.76 mg/g dry weight levels in steamed samples and 5.33 mg/g dry weight in autoclaved samples. Nano-ZnO1 and Nano-ZnO2 exhibited a more significant decrease in phytic acid levels than the Control. Autoclaving caused a more significant decrease (5.24mg/g dry weight) than steaming process (6.45mg/g dry weight) in Nano-ZnO1. The phytic acid reduction was also more pronounced with autoclaved samples (6.37mg/g dry weight) than steamed samples (7.24mg/g dry weight) in Nano-ZnO2 samples. Phytate reduction has been attributed to producing free radicals by heat treatment (Corzo-Ríos *et al*., 2022; Margier *et al*., 2018).

Table 3. Effects of Steaming, Autoclaving, and Nano-Zinc Oxide Foliar Application on total phenolic compound, phytic acid, and antioxidant activity of Faba Bean Seeds.

Treatments	TPC (mg GAE/100g)	Phytic acid (mg/g)	DPPH (mgTrolox /100 g)	ABTS (mgTrolox /100 g)
		Control		
Raw	169.67 ± 3.61^{a}	9.69 ± 0.13^{a}	626.34 ±3.05^{f}	255.27 ±2.16^{d}
Steamed	135.91 ± 1.67^{b}	6.76 ± 0.34^{c}	831.07 ±3.162^{a}	273.83 ±3.84^{ab}
Autoclaved	119.8 ± 1.75^{c}	5.33 ± 0.40^{e}	812.14 ±2.48^{b}	265.97 ±1.98^{bc}
		Nano-ZnO1		
Raw	167.88 ± 3.61^{a}	8.58 ± 0.23^{b}	641.70 ±3.11^{e}	253.80 ±2.61^{d}
Steamed	131.1 ± 1.85^{b}	6.45 ± 0.21^{d}	801.46 ±1.43^{c}	275.75 ±1.78^{a}
Autoclaved	117.1 ± 1.34^{c}	5.24 ± 0.30^{e}	735.62 ±3.46^{d}	266.54 ±2.34^{bc}
		Nano-ZnO2		
Raw	162.53 ±1.84^{a}	8.67 ± 0.35^{b}	642.52 ±2.01^{e}	254.78 ±1.91^{d}
Steamed	132.51 ±1.27^{b}	7.24 ± 0.23^{c}	833.06 ±2.53^{a}	274.87 ±2.82^{a}
Autoclaved	118.91 ±1.69^{c}	6.37 ± 0.31^{d}	803.91 ±3.08b^{c}	264.29 ±3.97^{c}

Values are presented as means ± SD. Different lowercase letters within a column indicate significant differences at $p \leq 0.05$.

The DPPH and ABTS radical scavenging abilities of the Faba beans were evaluated to determine the effect of thermal treatment on their antioxidant activity, as shown in Table 3.

Steaming process significantly enhanced the DPPH activity and ABTS compared with raw and autoclaved beans, indicating that steaming improves the free radical scavenging capacity. This enhancement suggests that steaming process preserve or activate antioxidant compounds in Faba beans. Also, these compounds could be generated due to the degradation of macromolecules or the formation of new compounds during the heating processes. Also, Siah *et al*. (2014) suggested that the antioxidant properties could result from the production of aglycones through the degradation of flavonoid glucosides. Autoclaving also improved the DPPH activity compared to

raw beans but was slightly less effective than steaming. This indicates that the cooking process may significantly reduce the effects of antinutritional compounds in foods and control the availability of nutrients. (ElMaki *et al*., 2007).

Particle size distribution.

The particle size of Faba beans flour affects the surface area available for digestive enzymes to interact with proteins, thereby influencing protein digestibility. (Paz-Yépez *et al*., 2019). Table 4 shows the particle size distribution, defined by three percentiles (10th, 50th, and 90th). These percentiles represent the particle size at which 10%, 50%, and 90% of the sample volume are present. There are no significant differences in the control samples at the three levels (10%, 50%, and 90%). Steaming and autoclaving process resulted in a minor shift in particle size distribution, marked by an increase in the 50th percentile and a decrease in the 90th percentile. This shift in particle size could be attributed to protein aggregation. The findings align with those of Martineau-Côté *et al*. (2023), indicating that boiling predominantly leads to a shift in the distribution, enlarging the median particle size while reducing the sizes of larger particles.

Table 4. The particle size distribution of activity in raw, Steamed, and Autoclaved Faba bean flour.

		Particle Size Distribution		
Treatments		**d(0.1) (µm)**	**d(0.5) (µm)**	**d(0.9)(µm)**
Control	Raw	7.54± 0.17^{bc}	24.32 ± 0.46^{f}	136.23± 0.87^{a}
	Steamed	7.57± 0.20^{bc}	36.81± 0.57^{c}	117.47± 0.75^{c}
	Autoclaved	8.15± 0.17^{a}	41.05± 0.65^{a}	114.31± 0.89^{d}
Nano-ZnO1	Raw	7.30± 0.15^{c}	25.18± 0.58^{f}	135.76± 0.93^{a}
	Steamed	7.56± 0.22^{bc}	36.43± 0.52^{c}	119.57± 0.91^{b}
	Autoclaved	7.85± 0.11^{ab}	37.71± 0.63b	118.53± 0.85^{bc}
Nano-ZnO2	Raw	7.49± 0.19^{bc}	24.84± 0,74^{f}	136.53± 0.56^{a}
	Steamed	7.60± 0.24^{bc}	30.23± 0.49^{e}	118.61± 0.75^{bc}
	Autoclaved	7.75± 0.23^{b}	32.37± 0.62^{d}	117.58± 0.94^{c}

Values are presented as means ± SD. Different lowercase letters within a column indicate significant differences at p ≤ 0.05.

Hunter color properties.

Table 5 presents the color properties (L, a, b values) of Faba beans flour under various treatments, including Control, Nano-ZnO1, and Nano-ZnO2. These treatments were applied in Raw, Steamed, and Autoclaved conditions. Heat treatments such as steaming and autoclaving significantly impact the lightness (L) and color components (a and b) of Faba beans. The lightness (L) generally increases with heat treatment, with autoclaved samples showing the highest values. The red/green component (a) decreases significantly with steaming and autoclaving, suggesting the degradation of red pigments under high temperatures(Medhe *et al*., 2019; Shevkani *et al*., 2015).

The yellow/blue component (b) tends to increase slightly with steaming, indicating the release or transformation of yellow compounds such as carotenoids during heat treatment (Sharma *et al*., 2015). Nano-ZnO1 samples show higher lightness (L) and a lower red/green component (a) compared to controls. The yellow/blue component (b) exhibits variability across treatments. Steamed Nano-ZnO1sample have the highest b values (14.68). Conversely, autoclaved samples show relatively stable b values. The presence and concentration of flavonoids, such as flavonols, anthocyanins, and condensed tannins, influence the color variation in fresh and processed beans and their flours (Sharma *et al*., 2015).

The chroma value indicates the intensity of a color in a sample, while the hue angle measures an average person's capacity to recognize that color. No significant differences were observed in the chroma values for the raw samples. The steamed Nano-ZnO2 sample had the highest chroma value (15.08), and the autoclaved Nano-ZnO2 sample recorded the highest hue angle (72.35).

Table 5. Color properties of raw, Steamed, and Autoclaved Faba bean flour.

Treatments	***L***	***a***	***b***	**Croma Value**	**Hue angle**
Control					
Raw	64.93 ± 0.34^{e}	7.13 ± 0.10^{ab}	12.23 ± 0.19^{b}	14.16 ± 0.21^{bc}	59.73 ± 0.09^{e}
Steamed	73.55 ± 0.86^{ab}	6.68 ± 0.21^{ab}	12.88 ± 0.29^{b}	14.32 ± 0.34^{bc}	62.58 ± 1.11^{d}
Autoclaved	74.56 ± 0.66^{ab}	6.18 ± 0.11^{b}	12.49 ± 0.36^{b}	13.62 ± 0.13^{c}	63.67 ± 1.03^{cd}
Nano-ZnO1					
Raw	68.48 ± 0.52^{c}	7.64 ± 0.88^{a}	12.87 ± 0.30^{b}	13.86 ± 0.23^{bc}	59.56 ± 0.24^{e}
Steamed	71.43 ± 1.13^{b}	5.56 ± 0.31^{c}	14.68 ± 0.55^{a}	14.51 ± 0.28^{ab}	63.91 ± 0.83^{c}
Autoclaved	74.30 ± 3.38^{ab}	5.16 ± 0.26^{d}	14.17 ± 0.26^{a}	13.94 ± 0.58^{bc}	70.07 ± 0.75^{b}
Nano-ZnO2					
Raw	67.36 ± 1.60^{d}	7.78 ± 0.44^{a}	12.09 ± 0.65^{b}	13.94 ± 0.38^{bc}	59.90 ± 0.37^{e}
Steamed	72.39 ± 0.72^{ab}	4.66 ± 0.12^{e}	12.23 ± 0.19^{b}	15.08 ± 0.33^{a}	71.07 ± 0.77^{b}
Autoclaved	76.55 ± 2.85^{a}	4.32 ± 0.40^{f}	12.88 ± 0.29^{b}	13.79 ± 0.23^{c}	72.35 ± 0.26^{a}

Values are presented as means ± SD. Different lowercase letters within a column indicate significant differences at p ≤ 0.05.

FTIR analysis of flour

FTIR spectra for raw, steamed, and autoclaved Faba bean samples are shown in Fig.1. In the control sample, the broad peak around 3567 cm^{-1} corresponds to O-H stretching vibrations, indicating the presence of hydroxyl groups. The peak around 3289cm^{-1} corresponds to N-H stretching vibrations, indicative of proteins or amino groups. The absorption at 1489 cm^{-1} may correspond to C-H bending vibrations in carbohydrates or other organic compounds. Steamed sample shifted peaks at 3563 cm^{-1} indicate O-H stretching, which is slightly shifted compared to the Control (Handa *et al*., 2017).

Similarly, peaks at 3234 cm^{-1} suggest N-H stretching, also slightly shifted compared to the Control. The Amide II band, observed at 1526 cm^-1, indicates N-H bending and C-N stretching in proteins, suggesting structural changes that may occur due to steaming. Autoclaved samples showed further shifted peaks at 3664 cm^{-1}, indicative of O-H stretching, with a more pronounced shift than the steamed sample. At 3247 cm^{-1}, N-H stretching suggests additional structural changes. Moreover, the presence of an Amide II band at 1506 cm^{-1} further supports the significant structural changes in proteins due to autoclaving.

The shifts in the O-H and N-H stretching bands in steamed and autoclaved samples suggest hydrogen bonding and protein structure changes. These changes are more pronounced in the autoclaved sample, indicating a more

significant alteration of the molecular structure due to the intense thermal treatment. The presence and shifts of the Amide I and II bands (around 1653 cm^{-1}, 1526 cm^{-1}, and 1506 cm^{-1}) reflect alterations in the protein secondary structure. Steaming and autoclaving induce these changes, with autoclaving having a more substantial impact. The changes in peaks around 1489 cm^{-1} in the Control and their absence or shifts in treated samples indicate modifications in carbohydrate or other organic compounds due to thermal treatments. These molecular changes could be correlated with the enhanced protein digestibility and bioaccessibility observed in steamed and autoclaved samples. Kumar *et al.* (2022) Mohamed observed that pressure-cooked Faba beans showed a slight difference in the protein region (1600 -1700– and 1534 - 1570 cm−1) compared to raw Faba beans, which was attributed to protein degradation during processing treatments.

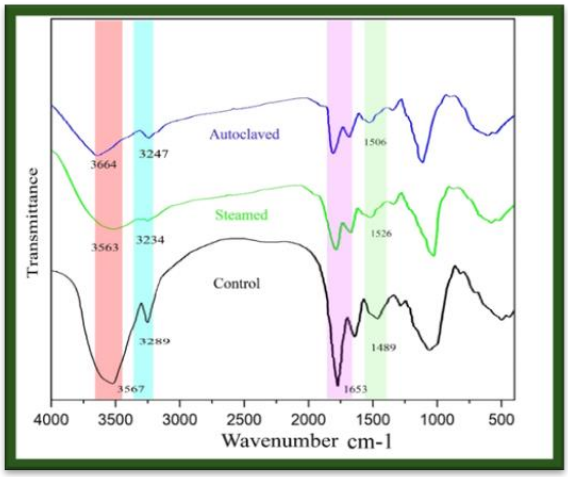

Fig.1. FTIR spectra of raw, Steamed, and Autoclaved Faba bean.

In vitro Digestibility of Protein (IVPB)

Legume seeds are a primary source of plant-based protein; however, their biological value depends on the composition and digestibility of the protein amino acids (Corzo-Ríos *et al*., 2022). Fig.2A shows the IVPB values from percentages of Faba bean varieties under different treatments: raw, steamed, and autoclaved. The raw Faba samples across all treatments (Control, Nano-ZnO1, and Nano-ZnO2) showed similar IVPB values, around 74.77-74.89%. No significant difference was observed among the raw Faba samples treated with different concentrations of Nano-ZnO. Steaming process increases the IVPB compared to raw Faba samples, with the values of 75.25% to 75.89%. The Control and Nano-ZnO treated samples show comparable digestibility values, indicating that steaming enhances digestibility, but Nano-ZnO addition does not significantly impact it. Autoclaving process further increases the protein digestibility, with values ranging from 80.53% to 80.83%. The differences between the Nano-ZnO-treated samples and the Control are not statistically significant, indicating that autoclaving significantly enhances protein digestibility regardless of Nano-ZnO treatment. Similar findings by Luo and Xie (2013) suggested that the IVPB of raw Vicia Faba was 73%, which increased to 79–80% in beans after heat treatment. Corzo-Ríos *et al*. (2022) found that heat treatments enhance the digestibility of beans, with boiling showing the most improvement of 4–6% higher than raw beans and roasting also showing improvement of 2-3% higher than raw beans.

The data indicates that thermal treatments (steaming and autoclaving) significantly enhance the IVPB of Faba bean. Autoclaving process is more effective than steaming process for Faba samples. Foliar application of Nano-ZnO does not significantly alter the protein digestibility compared to the Control, suggesting that thermal treatment is the primary factor in enhancing digestibility. The differences in digestibility among treatments may be attributed to changes in the cellular structure of the seeds, which occur as a defense response to heat exposure.(Zahir *et al*., 2021). Oliveira *et al*. (2017) researched the effects of cooking on different types of beans. They found that it causes physical and chemical changes in proteins, leading to differences in solubility, particularly in glutelins. The observed behavior may be attributed to the changes in the bound and unbound properties of the proteins produced by heat and the decrease or removal of substances that inhibit nutrient absorption. (Sánchez-Velázquez *et al*., 2021).

In Vitro Protein Bioaccessibility (IVPB)

Fig.2B presents the IVPB value of Faba bean varieties under different treatments: raw, steamed, and autoclaved. It includes a control group, and samples treated with Nano-ZnO1 and Nano-ZnO2. An in vitro bioaccessibility model provides a credible and efficient method for understanding the structural alterations in food consumed under simulated physiological conditions within the human gastrointestinal tract (Ayala-Rodríguez *et al*., 2022).

The raw samples across all treatments (Control, Nano-ZnO1, and Nano-ZnO2) show similar IVPB values, around 16.5-16.7%. No significant difference was observed among the raw samples treated with different concentrations of Nano-ZnO. Steaming increases IVPB compared to raw samples, with values ranging from 27.87% - 28.73%. The control group has the lowest IVPB among steamed samples, while NanoZ 1% shows the highest. However, the differences between the Control and NanoZ-treated samples are minimal and not statistically significant. Autoclaving further increases the IVPB, ranging from 32.15% to 33.71%. Similar to the steamed samples, the control sample and Nano-ZnO-treated samples show comparable bioaccessibility values, with the Control and Nano-ZnO1 samples slightly higher than the Nano-ZnO2 samples.

The thermal treatments (steaming and autoclaving) significantly enhance the protein bioaccessibility of the Faba bean. Both steaming and autoclaving improve bioaccessibility compared to raw samples, with autoclaving being the most effective method. The addition of Nano-ZnO, either at 1 $g \cdot L^{-1}$ or 2 $g \cdot L^{-1}$, does not substantially alter the bioaccessibility compared to the Control. However, a slight trend of decreased bioaccessibility exists with higher NanoZ concentration. This suggests that while Nano-ZnO addition has minimal impact, thermal processing is crucial in enhancing the protein bioaccessibility of Faba bean, implying that the protein fraction of the bean after heat treatment significantly increases its nutritional value. The observed phenomenon occurs due to the fact that proteins in their natural state adopt a certain structure specified by the precise arrangement of amino acids that make up the protein. This structure also limits the ability of proteolytic enzymes to interact with their target molecules (Joye, 2019).

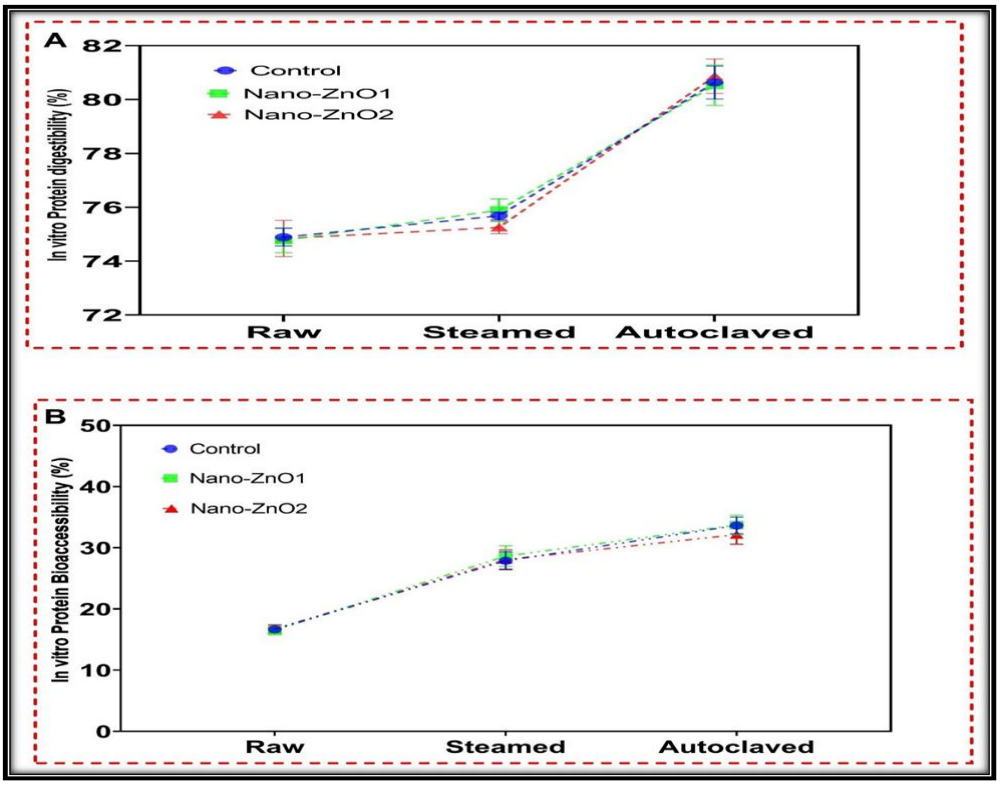

Fig. 2. Effects of Steaming, Autoclaving, and Nano-Zinc Oxide Foliar Application In vitro Protein digestibility (%) and In vitro Protein Bioaccessibility (%) of Faba Bean Seeds.

CONCLUSION

This study investigates the effects of thermal treatments (steaming and autoclaving) and nano-zinc oxide (Nano-ZnO) foliar applications on the nutritional properties of faba bean (*Vicia faba L.*) variety Sakha 1. The findings revealed that autoclaving significantly increases protein content and digestibility, with Nano-ZnO applications, particularly at 2 g·L-1, further enhancing these effects. Steamed beans treated with Nano-ZnO showed the highest protein levels. Both steaming and autoclaving also increased dietary fiber and ash content, with Nano-ZnO further increasing fiber levels. While thermal treatments generally reduced mineral content, Nano-ZnO at 2 g·L−1 helped retain higher mineral levels. These treatments reduced antinutritional compounds like phytic acid, improving nutrient bioavailability and the overall nutritional profile.

Using thermal treatments and Nano-ZnO foliar applications significantly improves the nutritional and functional characteristics of Faba beans. Future research should explore the environmental and economic implications of Nano-ZnO use to maximize its benefits for healthier food options.

ACKNOWLEDGMENT

The authors are most grateful for the technical support provided by the members of the Food Technology Research Institute, Agricultural Research Center. Egypt.

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تحسين قابلية هضم البروتين وتوافره الحيوي والاحتفاظ بالعناصر الغذائية في الفول باستخدام أكسيد الزنك النانوي والمعالجات الحرارية

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الملخص

أظهر البحث تأثير المعاملات الحرارية (بالبخار و بالاوتوكلاف،) على الخصائص الغذائية للفول البلدى (*Vicia faba L.*) صنف سخا ١ الذى تم رشة بتركيزات مختلفة بجزيئات أكسيد الزنك النانوية . يعتمد البحث علي تقييم التغيرات في التركيب الكيميائي، المعادن، وتجانس حجم الجزيئات، وخصائص اللون، وقابلية هضم البروتين، والتقييم الحيوي، ونشاط مضادات الأكسدة، ومحتوى المركبات الفينولية. المعالجة الحرارية، وخاصة بالاوتوكلاف، على تعزيز محتوى البروتين والألياف الغذائية والرماد بشكل كبير، حيث تظهر العينات المعامله بالاوتوكلاف أعلى قابلية لهضم البروتين وإمكانية الوصول البيولوجي للبروتين. زادت مستويات البروتين بشكل أكبر عند زياده نسبة أكسيد الزنك النانوية ، حيث أظهرت زيادة نسبة البروتين عند معاملة الفول بالبخار المعالجة بـ ٢ جرام / لترمن أكسيد الزنك النانوية. كما تعمل هذه المعاملات أيضًا على خفض مستويات حمض الفيتيك وتعزيز نشاط مضادات الأكسدة، وخاصة في العينات المطهيه بالبخار. على الرغم من أن المعاملات الحرارية تعمل عمومًا على خفض محتوى المعادن، الا ان الرش الورقى لاكسيد الزنك النانوية، وخاصةً عند ٢ جرام / لتر أظهر مستويات معدنية أعلى مقارنة بالعينات غير المعاملة بالطهى. كما يشير تحليل الـ FTIR إلى تغييرات واضحه في تركيب البروتينات بسبب المعاملات الحرارية، ويظهر تحليل اللون لعينات مسحوق الفول اختلافات كبيرة في مكونات السطوع واللون، كما تحسنت قابلية هضم البروتين معمليا بشكل كبير مع المعاملات الحرارية، حيث وصلت إلى ٨٠,٨٣٪ في العينات المعامله بالاوتوكلاف. تزداد أيضًا قابلية القيمه الحيوية التي وصل لها البروتين، مع أعلى قيمة بلغت ٣٣,٧١٪ في بذور الفول المعامله بالاوتوكلاف وجزيئات أكسيد الزنك النانوية.