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Effect of Zinc Enrichment on Growth and Nutritional Quality in Pea Sprouts

Yuan Lingyun[1], Wu Jian[2], Wang Chenggang[1], Liu Shan[1], Zhu Shidong[1,*]

[1]College of Horticulture, Anhui Agricultural University, Hefei 230061, China
[2]School of Information Engineering, Zhejiang A&F University, Zhejiang, China
*Corresponding author: z13605510842@qq.com

Abstract Zinc (Zn) is essential in plant nutrition and a fundamental component. Zn deficiency causes 450,000 deaths of children under 5 years of age every year. The slight lack of Zn leads to more diseases in humans, such as anorexia, loss of appetite, smell and taste failure. Thus, biofortification is used to provide the Zn nutritional status of plant food. The present study was conducted to investigate the effects of exogenous application of Zn by soaking and spraying on Zn contents, nutrition composition and antioxidant capacity in sprout. The results showed that plant growth was gradually increased from 10 to 50 mg L^{-1} of Zn; the nutrient values were also incremented by Zn of certain concentrations; and applied Zn treatment improved the total antioxidant capacity and contents of free amino acid. However, when Zn was over 50 mg L^{-1}, it resulted in reductions of nutrition value and antioxidant capacity, inhibiting the sprout growth. These results indicated that the optimal dose of Zn by soaking or spraying was 40-50 mg L^{-1}; and the impact of spraying was better than that of soaking. It was effective to enrich Zn content in certain extent by soaking or spraying, which could increase the nutrition value and antioxidant capacity as functional foods.

Keywords: *Zn richment, nutritional quality, antioxidant capacity, amino acid, sprout*

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1. Introduction

Zn is a ubiquitous trace element, which is a cofactor with diverse structural and catalytic functions in about 10% of all human proteins. Its major role is in the structure stabilization of a huge number of proteins, including signaling enzymes at all levels of cellar signal transduction and transcription factors [15]. WHO reports that about 2 billion (33 %) of the world population is affected by Zn deficiency, which causes 450,000 deaths of children under 5 years of age every year [3]. The slight lack of Zn leads to anorexia, loss of appetite, smell and taste failure, and other symptoms in humans. Thus, increasing Zn levels in crop will deliver more Zn to human body. Application of Zn was an effective strategy of biofortification to increase grain Zn concentration in wheat and rice [5,22], but information specific to pea is limited.

Pea (*Pisumsativum* L.) is a valuable supplement to cereals and other starchy food in the human diet due to excellent sources of protein and complex carbohydrates and good sources of minerals and vitamins [18]. Mineral malnutrition can be addressed by increasing the bioavailability of mineral elements in edible crops. Biofortification is a relatively new approach which aims to improve the nutritional status of the population by enhancing the micronutrient content of their staple plant food. Generally, agronomic biofortification through fertilization (its application to soils, seeds and/or leaves) helps to increase plant nutrient content without changing the plant's genetic makeup [23]. Thus, it is reasonable to enrich Zn in pea sprout by soaking seeds or spraying leaves in our study. Nowadays, the practice of sprouting is widely used to improve the nutritional value of crop seeds [14]. During germination, the content of nutritive factors such as vitamins and isoflavones and the bioavailabilities of trace elements and minerals increased [10]. It has been identified as an inexpensive and effective technology for improving the nutritional quality of crops.

The objective of present study is to investigate the optimum Zn application concentration and method (soaking or spraying) and demonstrate the effect of Zn biofortification on endogenous Zn contents, nutrition composition and antioxidant capacity in pea sprout, in order to provide a Zn-functional food and guide agronomic production.

2. Materials and Methods

2.1. Zn Enrichment

2.1.1. Soaking of Pea Seeds Treatment

The pea seeds were cleaned from all impurities including broken and diseased seeds. The seeds were soaked by submerging in Zn solution ($ZnSO_4$, Sangon Biotech, China) with each treatment (10mgL^{-1}, 20 mgL^{-1}, 30 mgL^{-1}, 40 mgL^{-1}, 50 mgL^{-1} and 60mgL^{-1}) in glass

container for 24 h at room temperature. The control was treated with water. After rinsing with water, the seeds were spread evenly on the trays, covering with the wet double-gauze to keep moist, and then placed in a controlled environment chamber at 28°C. They were sprayed with corresponding dose of Zn at internals of 8 h every day. When they grew to 13-15 cm, the shoots were obtained as the sample.

2.1.2. Spraying of Pea Sprouts Treatment

The growth condition of seeds was the same as2.1.1. After soaking seed with water, the seeds were sprayed with Zn solutions (10mgL^{-1}, 20 mgL^{-1}, 30 mgL^{-1}, 40 mgL^{-1}, 50 mgL^{-1} and 60mgL^{-1}) until the shoots grew to 13-15 cm. The control was treated with water. They were sprayed with different concentrations of Zn at internals of 8 h every day.

2.2. Analysis of Morphological

The height and stem diameter of the sprouts were determined using a ruler and vernier caliper, respectively. After shoots were washed with distilled water and wiped the water off, their fresh weights were measured.

2.3. Analysis of Zn Content

Pea sprouts were digested in a double acid mixture (HNO_3: $HClO_4$ = 2:1). Zinc in the digests was determined by automic absorption spectrophotometry (AAnalyst 800, Perkin Elmer, USA) [29].

2.4. Analysis of Crude Fibre Content

Crude fibre content was determined used method of acid and alkali mixture according to Sumczynski et al. (2015). 0.2g of dry sample was boiled for 30 min in 1.25% H_2SO_4, and then in 1.25% NaOH.

2.5. Analysis of Soluble Sugar Content

The shoots (0.02g) were soaked into 1 ml of distilled water, boiled for 30 min, and centrifuged at 5000×g for 4 min. The soluble sugars in the supernatant were quantified by the anthrone-sulfuric acid assay [30].

2.6. Analysis of Protein Content

Protein content was determined using the method of Coomassie brilliant blue. 0.5 g of fresh sample was grounded into homogenized with 5 ml of distilled water. 1.0 ml of supernatant was obtained after 10000×g centrifugal for 10 min. And then 5 ml of Coomassie brilliant blue G-250 was added and thoroughly mixed. The absorbance was measured at 595 nm.

2.7. Analysis of Chlorophyll Content

The chlorophyll was extracted from the third fully expanded leaf with a mixture containing acetone, ethanol, and water (4.5:4.5:1, v/v/v). The chlorophyll content was measured on a fresh weight basis according to a modified version of the method of Strain and Svec [24].

2.8. Analysis of Free Amino Acids

Free amino acids were assayed according to Aurisano et al.,(1995) with some modifications. The dried samples (0.5 g each) of shoot were homogenized with 2% sulphosalicylic amino acid [tissue to solution ratio 1:5 (w/v)] in a chilled pestle and mortar. The homogenate was adjusted to pH 2.0 with 0.02 M HCl and then centrifuged at 10,000×g for 15 min at 4°C. The amino acid content of the supernatant was determined with an amino acid analyzer (Hitachi 835-50, Japan).

2.9. Analysis of Antioxidant Capacity

2.9.1. Total Phenolic Content

According to the method of Randhir et al., [20], the total phenolic content was measured as galic acid equivalents from a gallic acid standard curve. The freeze-dried sprout powder (0.5g) was extracted and sonicated twice with 50 ml of 80% ethanol each time for 30 min. Then the mixture was centrifuged at 5,000×g for 20 min and the supernatant was used for the estimation. The absorbance was measured at 725 nm using a spectrophotometer.

2.9.2. Total Ascorbate (AsA) Content

Total AsA content were determined following Costa et al. [7] with slight modifications. Sample extract of 0.2 ml was activated with 60 mM DTT-ethanol and 0.2 M Na_2HPO_4, incubated for 10 min. The solution was then mixed with the reaction mixture containing 5% TCA, ethanol, 0.4% H_3PO_4-ethanol, 0.03% $FeCl_3$-ethanol and 0.5% BP-ethanol. The absorbance then recorded at 534 nm.

2.9.3. The Total Antioxidative Capacity (T-AOC) Analysis

The T-AOC was analyzed followed the total antioxidative capacity assay kit instructions from Nanjing Jiancheng Institute of Biotechnology. The antioxidants could reduce Fe^{3+} to Fe^{2+}, then Fe^{2+} and phenanthroline to form a stable Fe^{2+}-phenanthroline complex, which was measured through colorimetry to determine the antioxidant substances.

2.9.4. Activities of Antioxidant Enzymes

APX (EC 1.11.1.11) was assayed by monitoring the decrease rate of ascorbate oxidation at 290 nm (extinction coefficient = 2.8 mM^{-1} cm^{-1}) [17]. GR (EC 1.6.4.2) activity was determined at 340 nm using the molar extinction coefficient for NADPH (extinction coefficient = 6.2 mM^{-1} cm^{-1}) [8].

3. Statistical Analysis

The data were statistically analyzed using SAS software (SAS Institute, Cary, NC, USA), and Duncan's multiple range test at the $P < 0.05$ level of significance.

4. Results

4.1. Morphological Parameters

The fresh weight, plant height and stem diameter of pea sprouts were obviously improved by Zn applications through soaking or spraying (Table 1). These three growth parameters were gradually increased by soaking and spraying as increases of Zn concentrations. When soaking-

Zn applications reached 50 mg L^{-1}, the fresh weight, plant height and stem diameter were increased by 32.7%, 29.1% and 14.2% respectively, compared to control (0 mg L^{-1}). And these growth parameters by spraying-Zn were increased by 41.5%, 30.2% and 15.3% respectively. However, the plant growth were markedly decreased when Zn over 50 mg L^{-1} by both applications. According to Table 1, the spraying application had better effect on plant growth than soaking.

Table 1. Effects of soaking-Zinc and spraying-Zinc on growth parameters of pea sprouts

Treatments	Zn concentrations (mgL^{-1})	Fresh weight (g)	Plant height (cm)	Stem diameter (mm)
Soaking-Zn	0	0.52±0.011 e	11.52±0.11 f	1.76±0.09 d
	10	0.54±0.009 d	12.54±0.11 e	1.92±0.08 b
	20	0.55±0.004 cd	13.42±0.09 de	1.89±0.10 bc
	30	0.58±0.007 c	13.68±0.11 c	1.82±0.10 c
	40	0.64±0.013 b	14.43±0.09 b	1.96±0.97 b
	50	0.69±0.012 a	14.87±0.10 a	2.01±0.12 a
	60	0.61±0.009 cd	11.57±0.10 d	1.86±0.10 cd
Spraying-Zn	0	0.53±0.011 e	11.54±0.11 f	1.77±0.09 d
	10	0.65±0.015 d	13.07±0.09 e	1.84±0.09 c
	20	0.69±0.012 bc	13.67±0.98 d	1.92±0.12 b
	30	0.70±0.013 b	14.21±0.11 bc	1.85±0.08 ab
	40	0.71±0.018 b	14.75±0.08 b	1.90±0.09 bc
	50	0.75±0.015 ab	15.03±0.12 a	2.04±0.08 a
	60	0.61±0.017 d	10.88±0.09 f	1.83±0.10 c

Values represent the mean ± S.E. (n = 3). Letters indicate significant differences at $P<0.05$ according to Duncan's multiple range tests.

4.2. Zn Content

As the increasing of Zn applied by soaking or spraying, Zn content of sprout were continuously accumulated without peak in the present study (Figure 1). When Zn concentration reached 60 mg L^{-1}, Zn contents by soaking and spraying application were 168.56 mg kg^{-1} and 211.35 mg kg^{-1}, which was 357-fold and 448-fold to control (0 mg L^{-1}) respectively.

4.3. Chlorophylls Content

The chlorophylls content of pea sprouts were remarkably increased by Zn applications (10-60 mg L^{-1}) through soaking or spraying compared to control (0 mg L^{-1}) (Table 2). Chlorophylls content reached the highest level at 50 mg L^{-1} by soaking-Zn; the contents of chlorophyll a, b and a+b were increased by 3.01-fold, 1.95-fold and 2.59-fold respectively, compared to control. The spraying-Zn application had the best effect on chlorophyll a and a+b among all treatments at 40 mg L^{-1}, which were 5.81-fold and 2.51-fold to the control, respectively; the chlorophyll b reached the highest level at 50 mg L^{-1}.

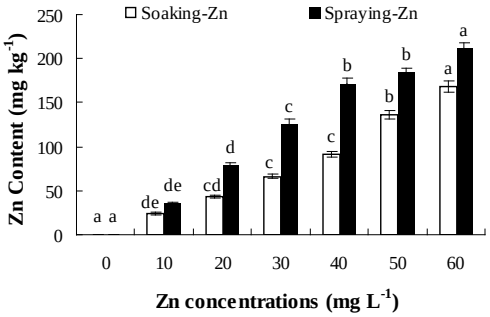

Figure 1. Effects of soaking-Zn and spraying-Zn on Zn content of pea sprouts. Values represent the mean ± S.E. (n = 3). Letters indicate significant differences at $P<0.05$ according to Duncan's multiple range tests

Table 2. Effects of soaking-Zinc and spraying-Zinc on chlorophylls content of pea sprouts

Treatments	Zn concentrations (mg L^{-1})	Chlorophyll *a* (mg g^{-1})	Chlorophyll *b* (mg g^{-1})	Chlorophyll *a+b* (mg g^{-1})
Soaking-Zn	0	0.82±0.02 e	0.38±0.01 d	1.27±0.08 e
	10	1.07±0.03 d	0.54±0.03 b	1.63±0.08 d
	20	1.43±0.03 cd	0.45±0.03 c	1.91±0.13 cd
	30	1.92±0.03 b	0.43±0.03 c	2.34±0.19 b
	40	2.12±0.02 ab	0.57±0.03 b	2.71±0.03 ab
	50	2.47±0.02 a	0.74±0.05 a	3.29±0.17 a
	60	1.88±0.04 bc	0.59±0.03 b	2.46±0.13 c
Spraying-Zn	0	0.81±0.02 f	0.40±0.01 d	1.25±0.76 e
	10	1.13±0.07 e	0.46±0.02 c	1.62±0.07 d
	20	1.53±0.16 d	0.53±0.05 b	1.98±0.10 c
	30	2.09±0.04 b	0.49±0.03 bc	2.58±0.22 ab
	40	2.61±0.06 a	0.55±0.03 b	3.13±0.16 a
	50	1.85±0.05 c	0.61±0.04 a	2.48±0.06 b
	60	1.62±0.02 d	0.42±0.04 cd	2.05±0.07 c

Values represent the mean ± S.E. (n = 3). Letters indicate significant differences at $P<0.05$ according to Duncan's multiple range tests.

4.4. Antioxidant capacity

4.4.1.Total Phenolic Content and T-AOC

Compared to control, the total phenolic content were significantly enhanced in pea sprouts by Zn applications of soaking or spraying, and spraying-Zn application had a better effect than soaking-Zn (Figure2 A). The phenolic content reached the peaks at 40 mg L^{-1} of Zn by soaking or spraying, increased by 3.8-fold and 4.9-fold respectively, compared to control. When Zn concentration was over 40 mg L^{-1}, the phenolic content was markedly declined in sprouts.

The T-AOC was significantly enhanced by soaking-Zn or spraying-Zn applications in sprouts (Figure 2 B). Similar to changes of total phenolic content, the T-AOC reached the highest level at 40 mg L^{-1} of Zn. Compared to control, T-AOC by soaking and spraying were increased by 25.3-fold time and 33.0-fold time respectively. The T-AOC of both applications was decreased as Zn concentrations over 40 mg L^{-1}.

4.4.2.The Activities of Antioxidant Enzymes

According to Figure 2, the APX activity was significantly enhanced by soaking or spraying applications as the increases of Zn. When Zn reached 50 mg L^{-1}, the APX activities of soaking and spraying were increased by 2.91-fold and 3.37-fold respectively, compared to control (Figure 2 C). The GR activities had the similar trend to the APX, increased by 7.24-fold and 8.11-fold through soaking and spraying respectively (Figure 2 D). When Zn was over 50 mg L^{-1}, both APX and GR activities were significantly decreased.

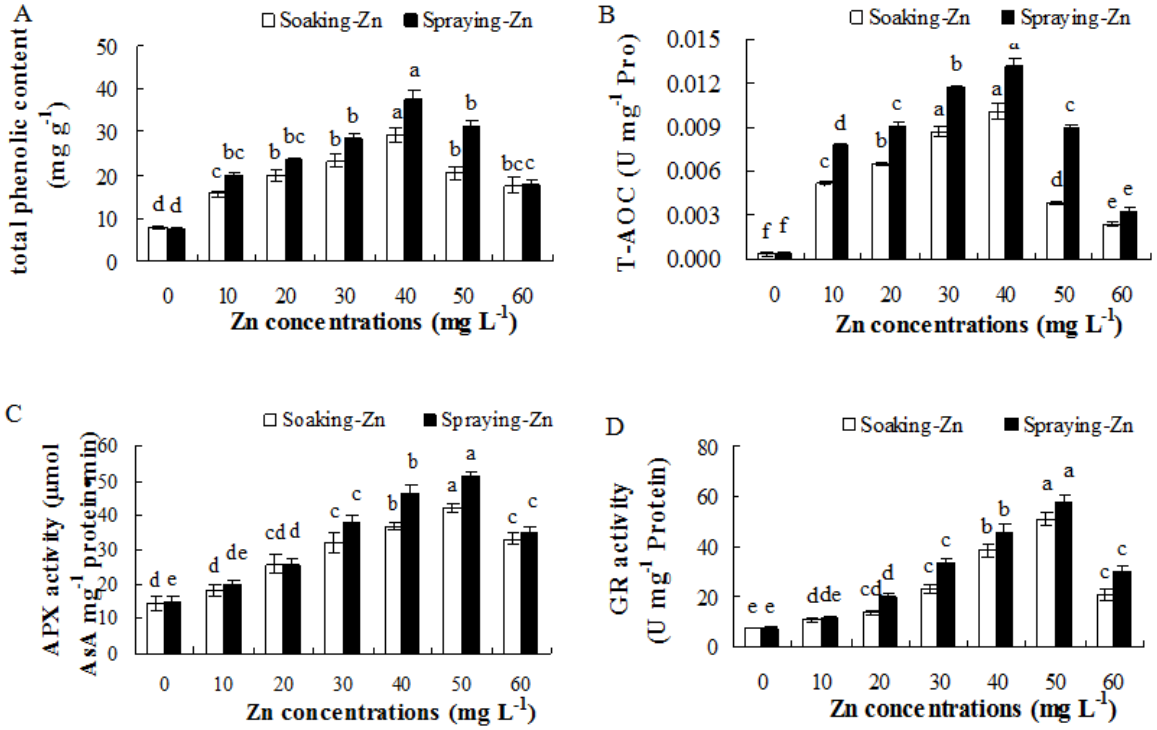

Figure 2. Effects of soaking-Zn and spraying-Zn on total phenolic content, T-AOC, APX activity and GR activity of pea sprouts. Values represent the mean ± S.E. (n = 3). Letters indicate significant differences at $P<0.05$ according to Duncan's multiple range tests

4.5. Nutrition Quality

The crude fibre contents were slightly increased by application of Zn of both treatments (Figure 3 A). There were no significant differences among all soaking-Zn treatments (10-60 mg L^{-1}). The crude fibre content of spraying-Zn had the highest level at 30 mg L^{-1} of Zn, which was increased by 8.78% compared to control.

As the increases of Zn concentrations between the 0 mg L^{-1} to 50 mg L^{-1}, the soluble sugar content of soaking or spraying applications was gradually increased in pea sprouts (Figure 3 B). When Zn reached 50 mg L^{-1}, the soluble sugar by soaking and spraying were 98.28 mg g^{-1} and 91.45 mg g^{-1}, which was increased by 48.9% and 35.5% to control (0 mg L^{-1}), respectively. The soluble sugar contents of both applications were dramatically declined at 60 mg L^{-1} of Zn compared to 50 mg L^{-1}.

The protein content had slight differences among all treatments in pea sprouts (Figure 3 C). The protein content reached the highest level at 30 mg L^{-1} of Zn by soaking or spraying-Zn, which were increased by 7.23% and 14.1% compared to control, respectively. When Zn concentrations was over 40 mg L^{-1}, the soluble sugar content was obviously declined.

The change pattern of AsA content by soaking or spraying was similar to the soluble sugar (Figure 3 D). The spraying-Zn had a better impact on the AsA content than that of soaking-Zn in pea sprouts. The AsA contents were significantly enhanced by both of two applications as the increases of Zn concentrations. The soluble sugar of soaking-Zn reached the peak at 50 mg L^{-1}, which was increased a 3.38-fold to control. And spraying-Zn of 40 mg L^{-1} application had the highest content of soluble sugar, increased a 3.26-fold time over control. When Zn concentrations were over 50 mg L^{-1} and 40 mg L^{-1} by

soaking and spraying respectively, the sugar contents were declined in pea sprouts.

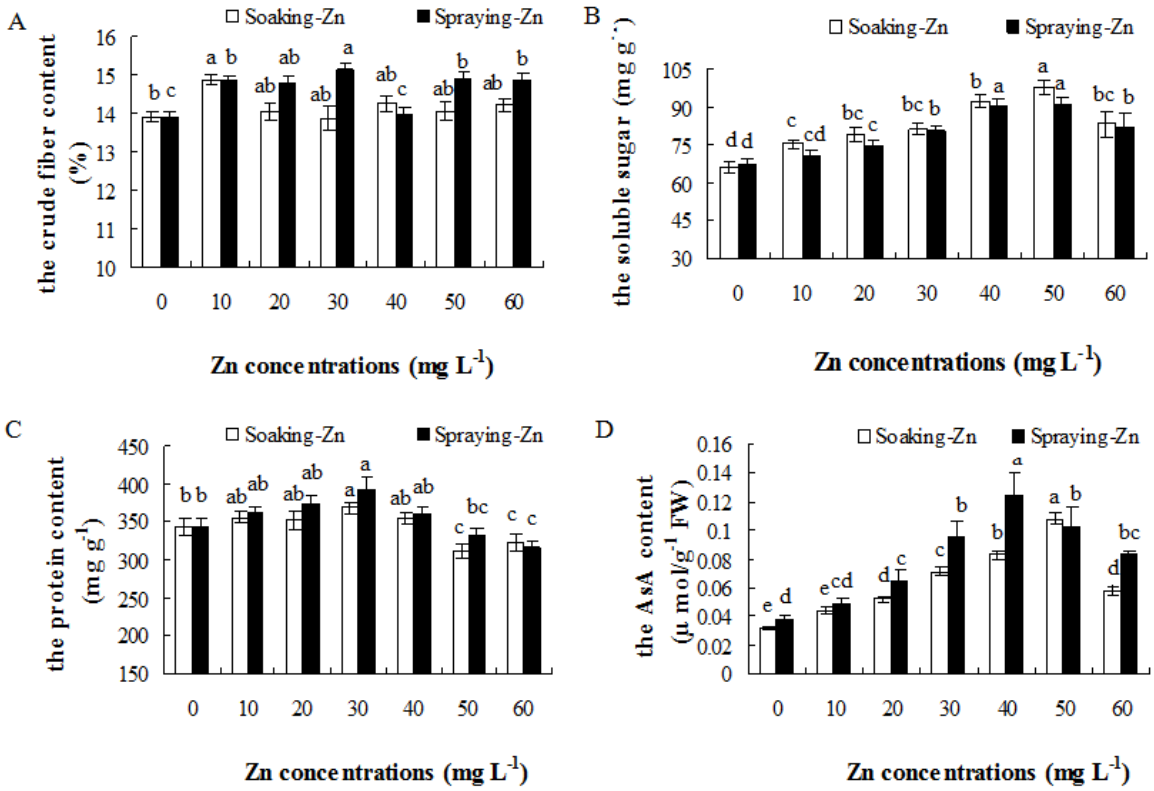

Figure 3. Effects of soaking-Zn and spraying-Zn on the contents of crude fiber, soluble sugar, protein and AsA of pea sprouts. Values represent the mean ± S.E. (n = 3). Letters indicate significant differences at $P<0.05$ according to Duncan's multiple range tests

Table 3. Effects of soaking-Zinc and spraying-Zinc on the amino acids content (mg 100g^{-1} DW) of pea sprouts

Soaking-Zn	0 mg L^{-1}	10 mg L^{-1}	20 mg L^{-1}	30 mg L^{-1}	40 mg L^{-1}	50 mg L^{-1}	60 mg L^{-1}
Aspartic acid	4.455	9.542	8.055	9.327	8.790	8.732	8.891
Threonine	0.460	0.939	0.969	0.944	0.909	0.987	0.981
Serine	0.443	0.941	0.982	0.942	0.936	1.003	0.972
Glutamic acid	6.422	2.968	3.074	2.999	2.937	3.245	3.048
Glycine	0.367	0.764	0.794	0.776	0.728	0.802	0.805
Alanine	0.718	1.524	1.510	1.648	1.515	1.563	1.621
Cysteine	0.143	0.220	0.226	0.234	0.219	0.229	0.246
Valine	0.596	1.500	1.574	1.528	1.786	1.525	1.538
Methionine	0.090	0.243	0.300	0.241	0.271	0.328	0.245
Isoleucine	0.346	0.724	0.791	0.732	0.746	0.808	0.748
Leucine	0.543	1.169	1.293	1.191	1.201	1.335	1.229
Tyrosine	0.202	0.612	0.706	0.686	0.653	0.640	0.672
Phenylalanine	0.589	1.191	1.301	1.221	1.296	1.296	1.245
Lysine	0.533	1.076	1.096	1.064	1.028	1.056	1.125
Histidine	0.298	0.652	0.638	0.658	0.612	0.640	0.660
Arginine	0.393	0.786	0.815	0.815	0.725	0.818	0.848
Proline	0.411	0.749	0.805	0.769	0.739	0.772	0.781
Total	17.007	25.601	24.928	25.774	25.092	25.778	25.653
Spraying-Zn	0 mg L^{-1}	10 mg L^{-1}	20 mg L^{-1}	30 mg L^{-1}	40 mg L^{-1}	50 mg L^{-1}	60 mg L^{-1}
Aspartic acid	4.525	8.719	8.252	8.838	8.713	8.701	8.331
Threonine	0.460	0.988	0.972	0.967	0.995	1.033	1.023
Serine	0.444	1.012	0.982	0.965	0.993	1.075	1.019
Glutamic acid	6.422	3.147	3.063	3.147	3.310	3.552	3.347
Glycine	0.367	0.786	0.772	0.758	0.830	0.857	0.815
Alanine	0.716	1.586	1.553	1.529	1.665	1.648	1.586
Cysteine	0.150	0.206	0.198	0.203	0.247	0.204	0.208
Valine	0.606	1.644	1.447	1.530	1.698	1.847	1.527
Methionine	0.090	0.292	0.269	0.312	0.223	0.320	0.263
Isoleucine	0.346	0.803	0.796	0.790	0.759	0.876	0.820
Leucine	0.542	1.322	1.299	1.272	1.257	1.478	1.384
Tyrosine	0.202	0.631	0.608	0.629	0.469	0.719	0.634
Phenylalanine	0.589	1.302	1.257	1.256	1.311	1.358	1.260
Lysine	0.535	1.100	1.071	1.033	1.179	1.217	1.165
Histidine	0.298	0.648	0.650	0.626	0.692	0.671	0.662
Arginine	0.391	0.819	0.756	0.795	0.883	0.907	0.844
Proline	0.417	0.768	0.761	0.752	0.780	0.821	0.791
Total	17.098	25.773	24.708	25.403	26.004	27.284	25.682

4.6. Free Amino Acids

Compared to control, the free amino acids content were significantly increased by application of Zn. According to Table 3, most of amino acids were increased to 2 or 3-fold to control. However, the glutamic acids were significantly declined in all treatments. The total free amino acids content got the highest level at 50 mg L^{-1} of Zn by both applications. Among other treatments of soaking-Zn or spraying-Zn, the total free amino acids had no obvious changes.

5. Discussions

Zn is a biologically essential trace element and is critical for cell growth, development, homeostasis, connective tissue growth, DNA synthesis, RNA transcription and cell activation, which is significant to human health as a functional element. In our study, it has been found that the Zn content, nutritional value and antioxidant capacity are considerably increased by application of Zn through spraying or soaking. Pea sprout could uptake and enrich Zn element, and in turn, an appropriate amount of Zn could also improve seed germination and sprout growth.

In the present study, the fresh weight, plant height and stem diameter in sprout by Zn application are higher than control. No reductions in plant biomass are associated with the increases of Zn concentrations until Zn is over 50 mg L^{-1}. This indicates that Zn, to be particular, promotes growth at optimal concentration but at higher level (>50 mg L^{-1}) restrains growth by interfering with the common metabolic activities of the plant, which is agreed with Mukhopadhyay et al. [16]. The higher Zn application by soaking or spraying might cause Zn toxicity. In general, the Zn concentration in mature leaf tissue is sufficient or normal if it lies between 25-150 mg kg^{-1} [11] and is excessive or toxic at 300 mg Zn kg^{-1} or more [28]. In our study, the Zn content in sprout is continuously accumulated along with the increases of Zn application of soaking or spraying. When application of Zn reached 60 mg L^{-1}, the Zn contents of sprout were 168.56 mg kg^{-1} and 211.35 mg kg^{-1}, respectively. Because the assimilating ability of sprout was lower than the mature leaves, we speculate that the higher Zn application (> 60 mgL^{-1}) results in remarkable Zn accumulation, thus causing Zn toxicity. Toxic effect of Zn, in this study, is evident from truncated growth and reduced biomass. The result also shows that spraying application causes more Zn accumulation than soaking in sprout, which indicates the effective of Zn absorption by spraying is better than soaking treatment.

In our study, by both applications, the total chlorophyll (Chlorophyll a and Chlorophyll b) contents in pea sprout are higher than that of control. The increased chlorophyll contents are due to increased Zn absorption that acts as a structural and catalytic component of proteins and enzymes and as a co-factor for normal development of pigment biosynthesis [26]. The higher chlorophyll content could promote photosynthesis, indirectly improving sprout biomass. However, the chlorophyll contents are significantly decreased when soaking-Zn concentration is over 50 mg kg^{-1}or spraying-Zn over 40 mg kg^{-1}. The decline of chlorophyll content in sprout exposed to higher concentration of Zn is believed to be due to inhibition of enzyme of chlorophyll biosynthetic such as δ-aminolaevulinic acid dehydratase and protochlorophyllide reductase [16]. Furthermore, the destruction of chlorophyll by higher Zn concentration could be due to peroxidation processes in the chloroplast membrane lipids by the ROS [21].

AsA, a critical component of AsA-GSH cycle, is the most abundant antioxidant in plant cells and sub-cellular compartments. In pea sprout, the differences existed between soaking-Zn and spraying-Zn. As the increasing of Zn concentration, the highest of AsA content appeared at 50 mg L^{-1} and 40 mg L^{-1} by soaking and spraying treatments respectively, and then both of them shows a decline tendency. The significant increases of AsA level with suitable range might be attributed to enhanced activities of APX and GR from AsA-GSH cycle in our study (Figure 4). However, higher Zn accumulation could stimulate the ROS production and cause oxidative stress in plant [16]. The oxidative damage is thought to be one of the major mechanisms involved in human chronic diseases, such as cancer and heart disease. Phenolic compounds are ubiquitous phytochemicals present in plant food with numerous biological activities including antioxidant properties. It has been shown that antioxidant-rich diets can reduce oxidative damage to DNA, thus preventing a critical step at the onset of carcinogenesis [31]. In our study, the total phenolic content and T-AOC capacity are continuously enhanced as the exogenous Zn are supplemented. The enhancement of antioxidant capacity is mostly associated with increased Zn content in pea sprout. In addition to enhanced antioxidant capacity, the protected role of Zn may be due to several factors: acting by stabilizing the cell membrane structure, maintaining an adequate level of metallothioneins (which are free radical scavengers), acting as an essential component of superoxide dismutase, acting as a protective agent for thiols, and in preventing the interaction between chemical groups with iron to form free radicals, as well as acting as an inhibitor of NADPH oxidase (effective scavenger of radicals) [6,19].

The crude fibre is a major component of plant cell wall, including cellulose, hemicellulose, ligin and cutin. The soaking-Zn treatment has no obvious effect on the crude fibre, while the crude fibre content is slightly increased by spraying treatment, which is beneficial that food containing crude fibre can promote bowel movements and digestion to a certain extent. Carbohydrate is the major end-product of photosynthetic carbon fixation and, with some exception, is the major transport form between plant cells, in contrast to many other groups of organisms [9]. The soluble sugar can behave in osmotic adjustment, or act as nutrient and metabolic signaling molecules to activate some specific transduction pathway [2]. In our study, the soluble sugar contents are gradually increased until application of Zn reached 50 mg L^{-1}. Zn is a structural part of carbonic anhydrase [4], which could improve carbon assimilation. Nevertheless, increased quantity of soluble sugar could be involved in the superior growth of the Zn-optimum plants and probably provided energy and osmolytes necessary for growth.

Decrease of soluble sugar by higher Zn application (>50 mg L^{-1}) could be the outcome of disturbed photosynthetic activity and modified sugar metabolism. The protein contents of sprout are slightly increased at 30

mg L^{-1} of Zn by both applications. There is no obvious change between the rest of treatments and control, excepting for higher Zn concentration (>50 mg L^{-1}). However, the analysis shows that most of the free amino acids are increased as the adding of Zn concentrations. Zn is required in a large number of proteins in organisms. It is often coordinated to the side chains of amino acids, such as cysteine and histidine, and aspartic acid [13]. Interestingly, in our study, we find that glutamic acid is remarkably decreased in all of Zn applications. The total free amino acid content at 50 mg L^{-1} is almost 1.5 times higher than control either by soaking or by spraying. The increment in the free amino acid content is favorable as the protein quality of the vegetable depends not only on its amino acid but also on the availability of these amino acids [12]. Aspartic acid is commonly found in the biosynthesis, which is the synthesis precursor of lysine, threonine, isoleucine and methionine. Its increase could contribute to the synthesis of amino acids. Among all of amino acids, proline is considered as a carbon and nitrogen source for cell growth, a stabilizer for membranes and also a free radical scavenger [27]. Increase of proline is believed to play positive roles in pea sprout growth. These results indicate that the high contents of necessary amino acids, such as threonine, valine, methione, isoleucine, leucine, tryptophane and phenylalanine, would provide high nutritional value.

6. Conclusion

In summary, this study demonstrates that application of Zn by soaking or spraying could effectively improve the biofortification of Zn. Accumulated Zn enhances the contents of chlorophylls, crude fibre, soluble sugar and antioxidant capacity, which is favorable to sprout growth. And applied Zn obviously increases the free amino acids level, providing more nutritional value. According to these parameters, we find that spraying treatment has a better impact on the nutrition value than soaking treatment. Among all of Zn concentrations, the optimum Zn concentration is 40 to 50 mg L^{-1} by soaking-Zn or spraying-Zn. These results provide strong theoretical support for practice production in functional foods.

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Conflict of Interests

The authors declare that they have no conflict of interest.

Abbreviations

APX, Ascorbate peroxidase; AsA, ascorbate; GR, Glutathione reductase; T-AOC, total antioxidant capacity; Zn, Zinc.

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