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Quality Characteristics of Germinated Radish Seeds Treated with Illite Clay

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Abstract: Radish (*Raphanus sativus* L.), a member of the cruciferous vegetable family, has been used as a medicinal food for a number of ailments. Radish contains a wide variety of phytochemicals that show antioxidative, antimutagenic, antiproliferative properties and functions in the induction of detoxification enzymes. The objective of this study was to examine the effect of illite treatment on the yield and nutrient value of radish sprouts. The yield, color appearance, and total mineral content of many of the illite-applied radish sprouts were improved compared to the control. Overall, lower concentrations of illite were found to be more appropriate to enhance the yield and nutritional values of radish sprouts.

Keywords: Amino Acid, Illite, Mineral, Radish Sprout, Yield

Introduction

Radish (*Raphanus sativus* L.) is a member of the cruciferous vegetable family that contains a variety of vegetables including broccoli, cauliflower, cabbage, and kale. Radishes have been used as medicinal foods for a number of ailments including liver dysfunction and poor digestion (Gutiérrez and Perez 2004; Lugasi et al. 2005; Shukla et al. 2011). Several studies have shown that radish or radish extracts contain antioxidant (Lugasi et al. 2005; Wang et al. 2010), antimutagenic (Nakamura et al. 2001), antiproliferative (Papi et al. 2008; Yamasaki et al. 2009; Beevi et al. 2010) properties, and play role in the induction of detoxification enzymes (Lee and Lee 2006; Hanlon et al. 2007).

The germination process is found to further improve the nutritional value of seeds (Paucar-Menacho et al. 2010) because it not only modifies the prevailing nutrients but also induces the release of new compounds (Kayahara et al. 2001). Lately, the use of seed sprouts as food has become popular in various parts of the Western world. A wide variety of sprouts can be found in the markets in which the *Cruciferae* family is well represented. Various studies have revealed that radish sprouts are very rich in health-promoting phytochemical constituents such as glucosinolates related to cancer prevention as well as having antioxidant properties (Barillari et al. 2005), phenolic compounds, and ascorbic acid in these vegetables (Takaya et al. 2003). An *in vivo* study demonstrated that Japanese radish sprouts are effective in alleviating hyperglycemia in diabetes cases and have potential benefits in the primary prevention of diabetes mellitus in animal models (Taniguchi et al. 2006). Isothiocyanates such as iberin and erucin, or the indole-3-carbinol present in broccoli and radish sprouts are found to have anticarcinogenic effects (Wagner et al. 2013). In addition, sulforaphene, obtained from glucoraphenin in radish sprouts, has drawn attention because of its potential anticancer effect (Pocasap et al. 2013).

Seed treatment before and/or during germination has been an extensively practiced technique to further augment the quality and nutrient value of sprouts. A number of experiments on seed treatment, using different biotic and/or abiotic factors, have been carried out to investigate their effects on the quality of sprouts (Lee et al. 2007; Yun et al. 2013; Algar et al. 2013; Chen and Chang 2015; Yang et al. 2015; Ferreira et al. 2019). Ferrous sulfate treatment during germination enhanced the iron content (Wei et al. 2013) and zinc sulfate improved the concentration and bioavailability of zinc (Wei et al. 2012) in the germinated brown rice. In a separate study, the use of zinc solution enhanced the sanitary and nutritional properties of durum wheat sprouts (Jribi et al. 2019). Calcium chloride treatment enhanced the yield and quality of soybean sprouts (Wang et al. 2016). The seed treatment with zinc sulfate solution increased the zinc content in soybean sprouts (Zou et al. 2014). Buckwheat sprouts grown with mineral-rich water were found to have elevated mineral and quercetin content (Pongrac et al. 2016). The selenite application in wheat, alfalfa, and sunflower sprout production resulted in high selenium content (Lintschinger et al. 2000). Low-pressure O_2 radio frequency discharge plasma irradiation induced the growth of radish sprouts (Kitazaki et al. 2012).

Regarding its effect on plant growth and development, Illite is a less-studied clay mineral that contains various

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mineral elements including potassium, calcium, magnesium, silicon, iron, and aluminum (Weaver 1965; Harder 1974; Lee et al. 2021). The objective of this study was to investigate the effect of illite treatment on the growth and quality of radish sprouts.

Materials and Methods

Radish seeds and sprout production

Radish (*Raphanus sativus* L.) seeds of cultivar Cheonga were purchased from a local market in Daegu, Korea.

One gram of intact seeds (for each treatment and replication) was washed with tap water and soaked in tap water containing different concentrations of illite or tap water alone for 6 h. The samples were named as control (IPR-0: seeds soaked in tap water alone), IPR-0.5 (seeds soaked in tap water containing 0.5% (w/v) illite powder), IPR-1 (seeds soaked in water containing 1.0% (w/v) illite powder), IPR-3 (seeds soaked in water containing 3.0% (w/v) illite powder) and IPR-5 (seeds soaked in water containing 5.0% (w/v) illite powder). After soaking for 6 h, the seeds were kept in 1-L plastic cups with a perforated base for the sprout cultivation. Radish sprouts were grown at room temperature 25±2°C for 36 h. The fresh sprouts were kept at –70°C and subjected to freeze-drying. The freeze-dried sprouts were ground into powder (Speed Rotor Mill, Model KT-02A) and put into airtight containers for subsequent analyses.

Measurement of germinated radish sprouts

The total fresh weight of germinated radish sprouts in each was recorded at the end of the germination period of 36 h.

Color value measurement

Values for L* (lightness: 100 score for white and 0 for black), a* (redness, + or greenness, -), and b* (yellowness, + or blueness, -) in dried samples were measured using a Chroma Meter (CR-300, Minolta Corp., Osaka, Japan). A Minolta calibration plate (YCIE = 94.5, XCIE = 0.3160, YCIE = 0.330) and a Hunter Lab standard plate (L* = 82.13, a* = -5.24, b* = -0.55) were used to standardize the instrument with D65 illuminant (Kim et al. 2014). The color was measured randomly on 3 areas of samples and the average was calculated.

Determination of mineral composition

The mineral content of the radish sprout was determined following the method described earlier (Skujins 1998). A fraction of the sample powder (0.5 g) was placed in a cup and treated with 15 mL of nitric acid. The solution was diluted with distilled water (2 mL). Mineral concentrations were determined using an inductively coupled plasma atomic emission spectrometer (ICP AES, Varian Vista Inc., Victoria, Australia).

Determination of amino acid profile

The amino acids profile was determined following a standard procedure (Je et al. 2005) with some modifications. One hundred milligrams of sprout powder was hydrolyzed with 6 N HCl (1 mL) in a sealed-vacuum ampoule at 110°C for 24 h. The HCl was removed from the hydrolyzed sample on a rotary evaporator and the volume was adjusted to 2.5 mL with 0.2 M sodium citrate buffer (pH 2.2). The sample was passed through a cartridge (C-18 Sep-Pak, Waters) and filtered through a 0.22 μm membrane filter (Millipore, Billerica). Amino acids were determined in an automatic amino acid analyzer (Biochrom-20, Pharmacia Biotech, Uppsala, Sweden).

Statistical analysis

Data were subjected to analysis of variance using Molecular Evolutionary Genetics Analysis (MEGA) software 4.0 (Analytical Software, Tucson, AZ, USA). Differences between means at $p \leq 0.05$ were analyzed using the Tukey test.

Results and Discussion

Sprout yield

Illite application significantly increased the yield of radish sprouts (Table 1). The highest sprout yield increment was found for IPR-1 (15.6%), followed by IPR-5 (8.3%) and IPR-3 (7.9%), compared to the control. The 3 and 5% illite solution treatment had significantly equal sprout yields.

Table 1. Effect of different concentrations of illite solutions on yield of germinated radish

	Sample [1)]				
	IPR-0	IPR-0.5	IPR-1	IPR-3	IPR-5
Total weight (g)	37.60±0.51[d 2)] (100.0%) [3)]	39.80±0.21[c] (105.9%)	43.45±0.31[a] (115.6%)	40.57±0.41[b] (107.9%)	40.73±0.32[b] (108.3%)

[1)] IPR-0 (control: seeds soaked in tap water), IPR-0.5 (seeds soaked in tap water containing 0.5% (w/v) illite powder), IPR-1 (seeds soaked in tap water containing 1.0% (w/v) illite powder), IPR-3 (seeds soaked in tap water containing 3.0% (w/v) illite powder) and IPR-5 (seeds soaked in tap water containing 5.0% (w/v) illite powder).
[2)] Values followed by different superscripts within a row indicate significant difference ($p<0.05$).
[3)] Percentage based on total weight of control. Values was expressed as mean ± standard deviation of triplicate replicates.

The influential role of potassium present in the illite solution might have increased the growth of illite-treated radish sprouts (Leigh and Wyn Jones 1984; Ahanger and Agarwal 2017). Potassium is found to have a role in enzyme regulation, cell elongation, and osmotic adjustment (Bhandal and Malik 1988). Minerals including calcium (Weaver 1965; Harder 1974; Lee et al. 2021) may affect the synthesis of plant growth regulators such as indoleacetic acid and gibberellin that could influence the production of radish sprouts (Wang et al. 2016).

Hunter's color value

Illite treatment significantly affected most of the color values in the different sprout samples (Table 2). The lightness and redness values of all sprout samples were decreased with illite treatment. However, mixed effects of illite were found in the yellowness of radish sprouts. The highest yellowness was found in IPR-0.5 (16.39) and the lowest in IPR-5 (14.72).

Table 2. Hunter's color value of germinated radish cultivated with different concentrations of illite treatment

Sample [1)]	Color value [2)]		
	L* (Lightness)	a* (Redness)	b* (Yellowness)
IPR-0	57.77±0.22[a 3)]	9.84±0.07[a]	15.66±0.09[b]
IPR-0.5	57.10±0.12[b]	9.27±0.04[c]	16.39±0.17[a]
IPR-1	56.60±0.32[c]	8.73±0.06[e]	15.72±0.10[b]
IPR-3	56.02±0.47[c]	8.94±0.12[d]	15.86±0.11[b]
IPR-5	54.97±0.33[d]	9.41±0.16[b]	14.72±0.06[c]

[1)] Samples are defined in Table 1.
[2)] L: lightness (100, white; 0, black), a: redness (-, green; +, red), b: yellowness (-, blue; +, yellow).
[3)] Values are mean±standard deviation of triplicate experiments. The values followed by the different letters in the same column are significantly different, according to Tukey test (p<0.05).

The color appearance of food products is a vital trait to increase the consumers' appeal to the product (Udomkun et al. 2018). Reduced lightness and redness could increase the quality of radish sprouts because these are some of the key features of sprouts.

Mineral content

Illite treatment remarkably increased the total mineral content of radish sprouts (Table 3). Similarly, except for Zn which was significantly unaffected, all individual minerals content was also improved with illite application. The highest total mineral content was found in IPR-1 (9668.09 mg/kg), followed by IPR-0.5 (9410.78 mg/kg), IPR-3 (9330.85 mg/kg), and IPR-5 (8893.79 mg/kg). Treatment of lower concentrations (0.5 and 1% w/v) of illite showed the higher total mineral content in radish sprouts.

Table 3. Mineral contents (mg/kg) of germinated radish cultivated by illite treatment

Element	Sample [1)]				
	IPR-0	IPR-0.5	IPR-1	IPR-3	IPR-5
K	4424.01±40.17[d 2)]	4877.05±52.22[ab]	4967.83±45.00[a]	4803.57±39.22[b]	4609.95±50.17[c]
Ca	1993.07±22.12[c]	2058.15±25.00[b]	2119.05±18.62[a]	2010.74±23.17[b]	1869.07±20.69[d]
Na	133.45±3.72[c]	115.14±4.52[d]	159.23±6.21[b]	180.19±3.66[a]	131.34±3.72[c]
Mg	2100.21±20.92[d]	2212.65±25.33[b]	2298.79±36.12[a]	2192.22±32.67[c]	2127.64±30.17[d]
Mn	9.36±0.02[c]	12.31±0.12[b]	13.01±0.79[b]	14.71±0.92[a]	15.15±1.12[a]
Cu	11.97±0.09[b]	20.54±0.92[a]	9.63±0.87[b]	9.37±0.77[b]	8.21±0.21[c]
Fe	43.86±1.11[e]	85.08±1.37[c]	68.76±0.96[d]	90.28±2.12[b]	103.58±2.16[a]
Zn	29.89±1.67[a]	29.86±1.98[a]	31.79±1.92[a]	29.77±1.22[a]	28.83±1.51[a]
Total	8745.82	9410.78	9668.09	9330.85	8893.79

[1)] Samples are defined in Table 1.
[2)] Values are mean±standard deviation of triplicate experiments. The values followed by the different letters in the same row are significantly different, according to Tukey test (p<0.05).

Seed treatment with mineral-rich substances and biofortification of germinating sprouts with different minerals is found to be a common practice. Zinc sulfate application in soybean sprouts (Xu et al. 2012; Zou et al. 2014), mineral-rich water treatment of tartary buckwheat and wheat sprouts (Pongrac et al. 2016), and selenium-treated cereal sprouts (Lintschinger et al. 2000) were found to contain elevated minerals. Illite treatment improved the nutritional value of radish sprouts by increasing the mineral contents because various minerals have different functions in the human body. Mg, K, and Ca are beneficial against hypertension (Houston and Harper 2008); Fe is advantageous in oxygen transport, energy metabolism, mitochondrial respiration, DNA synthesis, and cellular growth and differentiation (Ganz 2013). Illite treatment showed a great potential to increase the mineral content of radish sprouts.

Free amino acid content

Although the amount of essential amino acid was increased in IPR-1, illite treatment reduced the total free amino acid concentration in radish sprouts (Table 4). A total of 26 amino acids were detected, whereas 10 amino acids were not detectable in either sample.

Table 4. Free amino acid composition (mg/g of dry weight) of germinated radish cultivated with different concentrations of illite treatment

Amino acid	Sample [1)]				
	IPR-0	IPR-0.5	IPR-1	IPR-3	IPR-5
Essential amino acid					
L-Threonine	0.534 [2)]	0.521	0.580	0.552	0.540
L-Valine	0.765	0.771	0.847	0.781	0.797
L-Methionine	0.124	0.092	0.087	0.088	0.069
L-Isoleucine	0.401	0.421	0.445	0.431	0.410
L-Leucine	0.647	0.612	0.704	0.688	0.633
L-Phenylalanine	0.512	0.499	0.497	0.469	0.459
L-Lysine	0.629	0.609	0.557	0.504	0.459
L-Histidine	0.444	0.492	0.520	0.510	0.515
Sub-total	4.056	4.017	4.237	4.023	3.882
Non-essential amino acid					
L-Asparitic acid	0.505	0.327	0.291	0.339	0.304
L-Serine	0.866	0.809	0.796	0.782	0.748
L-Glutamic acid	1.177	0.921	0.727	0.620	0.577
Glycine	0.295	0.301	0.310	0.315	0.324
L-Alanine	2.307	1.994	1.934	1.882	1.787
L-Tyrosine	0.477	0.482	0.418	0.402	0.354
L-Arginine	1.251	1.116	1.001	0.920	0.826
Proline	1.590	1.502	1.462	1.455	1.443
Sub-total	8.468	7.452	6.939	6.715	6.363
Other amino acid					
O-Phospho-L-serine	ND[3)]	ND	ND	ND	ND
Taurine	ND	ND	ND	ND	ND
O-Phospho ethanol amine	ND	ND	ND	ND	ND
Urea	0.233	0.277	0.032	0.523	0.199
L-Sarcosine	0.031	0.030	0.026	0.029	0.031
L-α-Amino adipic acid	ND	ND	ND	ND	ND
L-Citrulline	0.006	0.005	0.002	0.004	0.003
L-α-Amino-n-butyric acid	0.027	0.029	0.055	0.052	0.055
L-Cystine	0.184	0.165	0.144	0.120	0.105
Cystathionine	0.010	ND	ND	ND	ND
β-Alanine	0.031	0.030	0.033	0.032	0.033
D,L-β-Amino isobutyric acid	0.017	0.016	0.013	0.014	0.013
γ-Amino-n-butyric acid	0.316	0.352	0.389	0.366	0.335
Ethanol amine	0.091	0.099	0.107	0.019	0.117
Hydroxylysine	ND	ND	ND	ND	ND
L-Ornithine	0.019	0.025	0.031	0.030	0.029
1-Methyl-L-histidine	ND	ND	ND	ND	ND
3-Methyl-L-histidine	ND	ND	ND	ND	ND
L-Anserine	ND	ND	ND	ND	ND
L-Carnosine	ND	ND	ND	ND	ND
Hydroxy proline	ND	ND	ND	ND	ND
Sub-total	0.965	1.028	0.832	1.189	0.920
Total free amino acid	13.489	12.497	12.008	11.927	11.165

[1)] Samples are defined in Table 1.
[2)] Values are mean of duplicate experiments.
[3)] ND: Not-detectable.

The decrease in the amount of amino acids in the samples with illite treatment might be due to illite stress and/or modification of seed proteins for sprout growth and synthesis of other bioactive compounds (Lisiewska et al. 2009). On the other hand, the increase in some of the amino acids might be due to calcium present in illite that may play a role in the activation of diamine oxidase activity and in increasing the content of some amino acids in the illite-treated sprouts (Wang et al. 2016).

Conclusions

The influence of illite on the growth and nutrient content of radish sprouts was investigated. The yield, color appearance, and total mineral content of many of the illite-applied radish sprouts were improved compared to the control. Overall, lower concentrations of illite were found to be more appropriate to enhance the yield and nutritional values of radish sprouts.

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