

Effect of Illite Treatment on the Yield and Quality Characteristics of Germinated Carrot Seeds

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Abstract: Carrot (*Daucus carota* L.) is an economically important vegetable crop that contains several health-promoting phytochemicals. Carrot seed extracts help in cardio-protection, muscle contraction regulation, regulating the total cholesterol and triglyceride high-density lipoprotein and very-low-density lipoproteins. The objective of this study was to examine the effect of carrot seed priming with different concentrations of illite solution. Although the yield of carrot sprouts was not significantly increased, the concentration of total mineral and total free amino acid of a few sprout samples were raised with illite treatment. Furthermore, the content of functional amino acids like γ -amino-n-butyric acid was improved in the illite-treated carrot sprouts. The results showed that illite treatment could offer a good option to enhance the quality of carrot sprouts.

Keywords: Amino Acid, Illite, Mineral, Carrot Sprout

Introduction

Carrot (*Daucus carota* L.), an economically important root vegetable, has gained popularity due to increased health consciousness of its nutritional value. Carrot is also known as a “good for the eyes” food owing to its high hydrocarbon carotenoids content, a precursor to vitamin A. Carrot is an important source of dietary fiber and natural antioxidants, including carotenoids, vitamins, minerals, and phenolic compounds (Nicolle et al. 2004; Arscott and Tanumihardjo 2010; Que et al. 2019). These phytochemicals function as free radical scavengers and inhibitors of prooxidative enzymes or external agents. Intake of carrot roots supplies plentiful biologically active substances which are reported to have a role in preventing diseases (Arscott and Tanumihardjo 2010; Que et al. 2019). Carrots contain a unique combination of three flavonoids: kaempferol, quercetin, and luteolin (Ching and Mohamed 2001; Lila 2004; Horbowicz et al. 2008). Bioactive polyacetylenes, such as falcarinol (also known as panaxynol), and falcarindiol are also found in carrots (Lund and White 1990). Falcarinol is supposed to stimulate cancer-fighting mechanisms in the human body. Daucoside and daucosin, sesquiterpenoids isolated from carrot seeds, have cytotoxic effects against human gastric cell lines (Ahmed et al. 2005; Fu et al. 2010).

Muralidharan et al. (Muralidharan et al. 2008) reported that carrot seed extracts help in maintaining membrane-bound enzymes associated with cardioprotection and muscle contraction regulation in rats. Similarly, rats fed with carrot seeds had reduced total cholesterol and

triglyceride high-density lipoprotein and very-low-density lipoproteins as compared to the control group. Aydin et al. (Aydın et al. 2010) found a liver membrane-stabilizing effect of carrot seed extract. The antioxidant potential of carrot seed extracts has also been documented (Rezaei-Moghadam et al. 2012; Singh et al. 2012).

Germination has become one of the economic ways to enhance the nutritional value of seeds (Paucar-Menacho et al. 2010). Germination not only transforms the prevalent nutrients but also favors the release of novel compounds (Kayahara et al. 2001). Production of sprouts as vegetables and salad has become popular because of simple and inexpensive technology requirements. The popularity of sprouts as food, which was mainly consumed in Asian countries, has gradually been increased in the Western world as well. Various cereal, legumes, and vegetable crop seeds have been used to produce sprouts. However, studies on carrot sprouts are greatly lacking. The objective of this study was to investigate the effect of illite, a clay mineral, treatment on the growth and quality of carrot sprouts.

Materials and Methods

Carrot seeds and sprouting

Carrot (*Daucus carota* L.) was purchased from a local market in Daegu, Korea.

Intact seeds (1 g) were separated (for each treatment and replication) and rinsed with tap water, followed by 6 h soaking the seeds in tap water alone or different concentrations of illite solution prepared in tap water. The sprout samples were denoted as control (CIP-0:



seeds soaked in tap water alone), CIP-0.5 (seeds soaked in tap water containing 0.5% (w/v) illite powder), CIP-1 (seeds soaked in water containing 1.0% (w/v) illite powder), CIP-3 (seeds soaked in water containing 3.0% (w/v) illite powder) and CIP-5 (seeds soaked in water containing 5.0% (w/v) illite powder). After 6 h of soaking, the seeds were transferred into 1-L plastic cups with a perforated base for the sprout cultivation. The sprouts were grown at room temperature ($25\pm 2^\circ\text{C}$) for 36 h. The freshly harvested sprouts were stored at -70°C for 24 h before freeze-drying. The freeze-dried sprouts were powdered using an electric motor (Speed Rotor Mill, Model KT-02A) and stored in airtight containers until subsequent analyses.

Measurement of germinated carrots

The total fresh weight of germinated carrot seeds was determined at the end of the germination period of 36 h by deducting the weight of 1-L cups from the gross weight of cups containing the germinating seeds.

Color value measurement

Hunter's color value of sprout powders was determined as L^* (lightness: 100 scores for white and 0 for black), a^* (redness, + or greenness, -), and b^* (yellowness, + or blueness, -) 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 on three areas of sample powders and the average was calculated.

Measurement of mineral content

The mineral concentration of carrot sprouts was measured according to a method described earlier (Skujins 1998) with some modifications. A half gram

of sprout powder was mixed with 10 mL of nitric acid. The mixture was diluted with distilled water (5 mL). Mineral concentrations were measured using an inductively coupled plasma atomic emission spectrometer (ICP AES, Varian Vista Inc., Victoria, Australia).

Determination of amino acid profile

The amino acids content was measured following a standard procedure described earlier (Je et al. 2005). Sprout powder (100 mg) was hydrolyzed with 6 N hydrochloric acid (1 mL) in a sealed-vacuum ampoule at 110°C for 24 h. The acid was removed from the hydrolyzed sample using a rotary evaporator and the volume was adjusted to 2.5 mL with 0.2 M sodium citrate buffer (pH 2.2). The sample mixture was passed through a cartridge (C-18 Sep-Pak, Waters) and filtered through a $0.22\ \mu\text{m}$ membrane filter (Millipore, Billerica). The concentration of amino acids was determined using an automatic amino acid analyzer (Biochrom-20, Pharmacia Biotech, Uppsala, Sweden).

Statistical analysis

Data were examined using analysis of variance in Molecular Evolutionary Genetics Analysis software 4.0 (Analytical Software, Tucson, AZ, USA). The significant differences between the treatments were identified using the Tukey test at $p \leq 0.05$.

Results and Discussion

Sprout yield

The effect of illite application was not significantly influential on the yield of carrot sprouts (Table 1). The sprout yield of CIP-0 (10.54 g) and CIP-1 (10.39 g) was statistically equal, whereas the other three treatments produced significantly lower (9.43–9.81 g) sprout yields compared to the control, CIP-0.

Table 1. Effect of different concentrations of illite treatment on the weight of germinated carrot seed

	Sample ¹⁾				
	CIP-0	CIP-0.5	CIP-1	CIP-3	CIP-5
Total weight (g)	10.54 \pm 0.31 ^{a2)} (100%) ³⁾	9.43 \pm 0.33 ^b (89.46%)	10.39 \pm 0.21 ^a (98.58%)	9.81 \pm 0.25 ^b (93.07%)	9.72 \pm 0.30 ^b (92.22%)

¹⁾ CIP-0 (control: seeds soaked in tap water), CIP-0.5 (seeds soaked in tap water containing 0.5% (w/v) illite powder), CIP-1 (seeds soaked in water containing 1.0% (w/v) illite powder), CIP-3 (seeds soaked in water containing 3.0% (w/v) illite powder) and CIP-5 (seeds soaked in water containing 5.0% (w/v) illite powder).

²⁾ Values are expressed as mean \pm standard deviation of triplicate replicates. Values followed by different superscripts within a row indicate significant difference ($p < 0.05$).

³⁾ Percentage in parentheses was calculated based on the relative weight of the control.

The mechanisms of the negative influential role of illite on the yield of the majority of the carrot sprout samples were not clear in this experiment. Generally, an illite solution is expected to enhance the growth of illite-treated sprouts (Leigh and Wyn Jones 1984; Ahanger and Agarwal 2017) because of potassium, which is found to have a role in enzyme regulation, cell elongation, and osmotic adjustment (Bhandal and Malik 1988). Moreover, calcium and other minerals (Weaver 1965; Harder 1974; Lee et al. 2021) are

supposed to have roles in the synthesis of plant growth regulators such as indoleacetic acid and gibberellin that influence sprout growth (Wang et al. 2016).

Hunter's color value

Illite treatment showed diverse effects on the color value of carrot sprouts (Table 2). The lightness value was increased in the higher concentrations of the illite solution, however, the redness and yellowness values did not show a consistent relationship with illite

concentration. The highest and lowest redness values were found in CIP-1 (3.19) and CIP-0.5 (2.76), respectively. The utmost yellowness value was

observed in CIP-0.5 (7.99) and the lowermost value in CIP-1 (7.45).

Table 2. Hunter's color value of germinated carrot seed powders treated with different concentrations of illite solution

Sample ¹⁾	Color value ²⁾		
	L* (Lightness)	a* (Redness)	b* (Yellowness)
CIP-0	43.69±0.15 ^{d3)}	2.87±0.01 ^b	7.61±0.01 ^c
CIP-0.5	43.78±0.02 ^d	2.76±0.05 ^c	7.99±0.02 ^a
CIP-1	44.19±0.12 ^b	3.19±0.04 ^a	7.45±0.03 ^d
CIP-3	44.68±0.20 ^a	2.86±0.03 ^b	7.82±0.05 ^b
CIP-5	44.47±0.08 ^c	2.89±0.05 ^b	7.67±0.07 ^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 reported as mean±standard deviation of triplicate experiments. The values followed by the different letters in the same column are significantly different ($p < 0.05$).

Color is a key characteristic of food products to attract consumers towards the product (Udomkun et al. 2018). The variations in the color values caused by the illite treatment could offer better options to produce good quality carrot sprouts.

Mineral content of germinated carrot seeds

Illite treatment increased the total mineral content in CIP-1 but reduced it in the other three treatments as compared to the control (Table 2). The highest total

mineral content was found for CIP-1 (20890.98 mg/kg) and the lowest was found in CIP-3 (15041.13 mg/kg). Although the amount of total mineral was reduced in many illite-treated sprouts, some individual minerals were increased e.g., Mn content was significantly high in CIP-5. The most abundant mineral was Ca (6022.23–7311.89 mg/kg), followed by K (5168.53–5850.01 mg/kg). Similarly, the most scarce mineral was Mn (20.27–34.87 mg/kg), followed by Zn (36.32–133.51 mg/kg).

Table 3. Mineral contents (mg/kg) of germinated carrot seeds treated with different concentrations of illite solution

Element	Sample ¹⁾				
	CIP-0	CIP-0.5	CIP-1	CIP-3	CIP-5
K	5850.01±282.41 ^a	5608.98±156.58 ^a	5408.30±271.20 ^{ab}	5168.53±117.77 ^b	5224.05±245.86 ^b
Ca	7311.89±139.34 ^a	6550.38±533.74 ^c	7133.19±133.92 ^a	6022.23±371.95 ^d	6711.30±39.22 ^b
Na	588.91±33.99 ^b	340.06±8.90 ^c	668.26±39.49 ^a	290.39±9.25 ^d	327.01±26.58 ^c
Mg	3688.41±37.79 ^a	3492.91±17.80 ^b	3481.34±15.45 ^b	3150.74±20.40 ^d	3304.92±14.43 ^c
Mn	20.27±0.23 ^c	24.87±0.07 ^c	29.79±0.25 ^b	30.01±0.28 ^b	34.87±0.16 ^a
Cu	380.86±1.60 ^b	32.57±0.16 ^d	1612.20±24.35 ^a	39.06±0.12 ^c	30.71±0.20 ^e
Fe	967.75±10.58 ^b	220.17±0.62	2424.39±27.82 ^a	303.85±2.95 ^c	299.46±0.69 ^c
Zn	58.84±0.40 ^b	36.92±0.24 ^c	133.51±0.13 ^a	36.32±0.17 ^d	36.43±0.20 ^{cd}
Total	18866.94	16306.86	20890.98	15041.13	15968.75

¹⁾ Samples are defined in Table 1.

²⁾ Values are expressed as mean±standard deviation of triplicate experiments. The values followed by the different letters in the same row are significantly different ($p < 0.05$).

Seed treatment with mineral-rich substances is expected to have a positive impact on the mineral content of the germinating sprouts. A number of studies are found to have adopted this approach. For instance, 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). Illite treatment could offer an easy approach to increase individual mineral elements and help enhance the nutritional value of carrot sprouts because different minerals have specific functions in the human body. Mg, K, and Ca are effective against hypertension (Houston and Harper 2008); Fe is beneficial in oxygen transport, energy metabolism, mitochondrial respiration, DNA synthesis, and cellular growth and differentiation (Ganz 2013).

Mn functions in carbohydrate metabolism, in coordination with enzymes in the body (Dias 2012a, b), and as a co-factor for the antioxidant enzyme, superoxide dismutase.

Free amino acid content of germinated carrot seeds

The amount of total amino acid was increased with illite treatment, except in the highest concentration (5% w/v) treatment (Table 4). Similarly, the essential amino acid was increased in CIP-0.5 (1.603 mg/g DW) and CIP-1 (1.654 mg/g DW) compared to the control (1.535 mg/g DW). A total of 27 amino acids were detected, whereas 10 amino acids were undetectable in either sample. The amount of functional amino acid like γ -amino-n-butyric acid (GABA) was increased with illite treatment.

Table 4. Free amino acid composition (mg/g of dry weight) of germinated carrot seeds treated with different concentrations of illite solution

Amino Acid	Sample ¹⁾				
	CIP-0	CIP-0.5	CIP-1	CIP-3	CIP-5
Essential Amino Acid					
L-Threonine	0.183 ²⁾	0.189	0.192	0.152	0.126
L-Valine	0.443	0.421	0.427	0.392	0.277
L-Methionine	0.044	0.051	0.056	0.047	0.036
L-Isoleucine	0.230	0.291	0.249	0.222	0.149
L-Leucine	0.217	0.219	0.243	0.231	0.157
L-Phenylalanine	0.111	0.110	0.130	0.102	0.082
L-Lysine	0.178	0.192	0.217	0.181	0.136
L-Histidine	0.129	0.130	0.140	0.123	0.086
Sub-total	1.535	1.603	1.654	1.450	1.049
Non-essential Amino Acid					
L-Aspartic acid	0.226	0.267	0.328	0.332	0.304
L-Serine	0.242	0.241	0.242	0.240	0.145
L-Glutamic acid	0.076	0.066	0.052	0.069	0.099
Glycine	0.060	0.069	0.095	0.082	0.064
L-Alanine	0.363	0.339	0.324	0.317	0.259
L-Tyrosine	0.066	0.067	0.076	0.065	0.035
L-Arginine	0.276	0.300	0.301	0.274	0.165
Proline	0.086	0.092	0.115	0.100	0.070
Sub-total	1.395	1.441	1.533	1.479	1.141
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	ND	ND	ND	ND	ND
L-Sarcosine	ND	ND	ND	ND	ND
L- α -Amino adipic acid	0.011	0.012	0.013	0.010	0.009
L-Citrulline	0.011	0.014	0.021	0.020	0.022
L- α -Amino-n-butyric acid	0.010	0.012	0.010	0.011	0.009
L-Cystine	0.018	0.019	0.021	0.015	0.011
Cystathionine	0.017	0.016	0.013	0.013	0.011
β -Alanine	0.010	0.010	0.025	0.020	0.023
D,L- β -Amino isobutyric acid	0.005	0.017	0.049	0.033	0.032
γ -Amino-n-butyric acid	0.236	0.277	0.361	0.312	0.281
Ethanol amine	0.037	0.040	0.045	0.043	0.027
Hydroxylysine	ND	ND	ND	ND	ND
L-Ornithine	0.003	0.004	0.008	0.005	0.006
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	0.012	0.011	0.012	0.009	0.007
Sub-total	0.370	0.432	0.578	0.491	0.438
Total Free Amino Acid	3.300	3.476	3.763	3.420	2.628

¹⁾ Samples are defined in Table 1.

²⁾ Values are expressed as mean of duplicate experiments.

³⁾ ND: Not-detectable.

The essential amino acids cannot be produced by an organism at the required rate, and thus must be supplied externally. The decrease in the amount of amino acids, especially in the sprouts samples with higher concentrations of illite treatment, could have been resulted 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). Amino acids GABA and glycine are related to learning and memory enhancement; stroke and neurodegenerative disease control; anxiety, sedation, and anticonvulsant relief;

and muscle relaxation functions (Mody et al. 1994; Oh and Oh 2004). The GABA-rich foods are regarded as beneficial for regulating blood cholesterol and pressure, decreasing insomnia and depression, and relieving pain (Dhakal et al. 2012). GABA regulated blood pressure and inhibited sleeplessness and autonomic disorder observed during the menopausal or presenile period (Okada et al. 2000) and for controlling diabetes (Reeds 2000).

Conclusions

The effect of illite treatment on the yield and quality of carrot sprouts was studied. The yield of carrot sprouts was not significantly increased with illite treatment. However, the concentration of total mineral and total free amino acid of a few sprout samples was elevated with illite treatment. Moreover, the amount of functional amino acid like GABA was increased in the illite-treated carrot sprouts. The overall results indicated that illite treatment could be adopted to enhance the quality of carrot sprouts.

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