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Effect of Cerium Oxide Nanoparticles on the Quality of Rice (*Oryza sativa* L.) Grains

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Supporting Information

ABSTRACT: Despite the remarkable number of publications on the interaction of engineered nanoparticles (ENPs) with plants, knowledge of the implications of ENPs in the nutritional value of food crops is still limited. This research was performed to study the quality of rice grains harvested from plants grown in soil treated with cerium oxide nanoparticles ($n\mathrm{CeO_2}$). Three rice varieties (high, medium, and low amylose) were cultivated to full maturity in soil amended with $n\mathrm{CeO_2}$ at 0 and 500 mg $\mathrm{kg^{-1}}$ soil. Ce accumulation, nutrient content, antioxidant property, and nutritional quality of the rice grains were evaluated. Results showed that rice grains from $n\mathrm{CeO_2}$-treated plants had less Fe, S, prolamin, glutelin, lauric and valeric acids, and starch. Moreover, the $n\mathrm{CeO_2}$ reduced in grains all antioxidant values, except flavonoids. Medium- and low-amylose varieties accumulated more Ce in grains than the high-amylose variety, but the grain quality of the medium-amylose variety showed higher sensitivity to the $n\mathrm{CeO_2}$ treatment. These results indicate that $n\mathrm{CeO_2}$ could compromise the quality of rice. To the authors' knowledge, this is the first report on the effects $n\mathrm{CeO_2}$ on rice grain quality.

KEYWORDS: *antioxidant capacity, cerium oxide nanoparticles, grain quality, Oryza sativa, nutritional value*

INTRODUCTION

The production of engineered nanoparticles (ENPs) has rocketed to an extent that the environmental contamination and exposure to ENPs is a growing concern.[1] Cerium oxide nanoparticles ($n\mathrm{CeO_2}$) are heavily used in applications such as chemical mechanical planarization, fuel catalysis, UV coatings, and paints, with a conservative annual global production estimate of 1000 tonnes.[2] The $n\mathrm{CeO_2}$ are stable in a range of environmental media,[3] and they have been found, mostly, in nanoparticulate form in different food crops.[4−6] Reports indicate that $n\mathrm{CeO_2}$ induced physiological changes in soil-grown soybean (*Glycine max* (L.) Merr.) and corn (*Zea mays* L.).[5,7] However, fundamental questions remain on how $n\mathrm{CeO_2}$ affect the quality of food crops.

Rice (*Oryza sativa* L.) is an important food crop feeding more than half of the world's population.[8] It is more valuable than corn (*Z. mays* L.) and wheat (*Triticum aestivum* L.) for human nutrition because it can provide superior energy per hectare and support more people per unit of land.[9] The effects of abiotic stresses on the nutrient content,[10,11] antioxidant property (phenolic contents and radical scavenging ability),[12] and nutritional quality (starch, sugar, protein, and fatty acid contents)[13−15] of rice have been studied. Studies also revealed that drought affects the protein and starch syntheses and carbohydrate metabolism in developing rice grain.[16] As the environmental release of ENPs is a concern, it is imperative to investigate their effects on the quality of a major agricultural crop such as rice.

Studies have shown that ENPs influence the elemental concentrations,[17−19] phenolic content and radical scavenging ability,[20−22] protein levels,[20,23−25] and carbohydrate contents in plants.[22,24,26,27] However, these studies have been performed at early growth stages and short exposure time; thus, the effect of ENPs on the quality of fruits or grains is still unknown.

The impacts of ENPs on the quality of fruits and seeds harvested from plants cultivated to full maturity are increasingly being investigated. Studies showed that *n*Au altered the total and reducing sugars and oil contents in seeds harvested from mustard.[28] Others have shown that *n*Ag elevated the total soluble solids in fruits of cucumber (*Cucumis sativus* L.),[29] but did not change the polyphenol contents in borage (*Borago officinalis* L.).[30] Recently, fullerol [$\mathrm{C_{60}(OH)_{20}}$] has been shown to improve the phytomedicine contents of fruits from soil-grown bitter melon (*Momordica charantia* Descourt.).[31] However, studies on the nutritional value of the edible portions of plants grown until full maturity in ENP-contaminated soil are still greatly lacking.

The varietal differences of rice play a role in the accumulation of toxicants in plant tissues. The heavy metals Cd, As, Cr, Cu, Hg, Ni, Pb, and Zn accumulated differently in grains of different rice varieties.[32−34] Studies have also confirmed the variety dependence in nutrient, phenolic, sugar, protein, and fatty acid contents in rice under different abiotic stresses.[10−15] The impacts of ENPs on plants through their life cycle have been investigated;[5,28−31] however, the effects of plant variety on ENP−plant interactions have yet to be understood.

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This study reports the interaction of nCeO$_2$ with three rice varieties (high, medium, and low amylose contents) grown until grain production in nCeO$_2$-amended soil. IR- and plasma-based spectroscopic techniques as well as biochemical assays were used to study the effects of nCeO$_2$ and rice varieties on Ce accumulation, nutrient content, antioxidant property, and nutritional quality of rice grains. This study should shed light on the effect of nCeO$_2$ on the nutritional profile of rice grains.

MATERIALS AND METHODS

Preparation of nCeO$_2$ Suspensions. The nCeO$_2$ (Meliorum Technologies, Rochester, NY, USA) were procured from the University of California Center for Environmental Implications of Nanotechnology. The nCeO$_2$ were previously characterized by Keller et al.[35] The nCeO$_2$ are rods with primary size of 8 ± 1 nm, particle size of 231 ± 16 nm in DI water, surface area of 93.8 m^2 g^{-1}, and 95.14% purity.[35] The amount of nCeO$_2$ necessary to prepare 500 mg nCeO$_2$ kg^{-1} soil was suspended in 400 mL of Millipore water by sonication in a water bath (Crest Ultrasonics, Trenton, NJ, USA) at 25 °C for 30 min with occasional stirring. The 500 mg nCeO$_2$ kg^{-1} treatment was chosen because in preliminary studies rice plants exposed to this concentration did not show phenotypical changes.

Pot Soil Preparation. Twelve plastic pots (24 cm diameter × 25 cm high) were filled with 5 kg of soil (Earthgro potting soil) previously mixed with nCeO$_2$ suspension to have a final concentration of 500 mg kg^{-1}. The soil was equilibrated for 3 days before rice seedlings were transplanted. Twelve pots were also prepared with untreated soil (control). The pots were irrigated with distilled water to maintain saturation for the duration of the experiment.

Rice Cultivation. Rice seeds from high-, medium-, and low-amylose varieties (Cheniere, Neptune, and 10AY004, respectively) were provided by Louisiana State University Agricultural Center (Baton Rouge, LA, USA). Thirty-day-old seedlings were transplanted into the pots and placed in a greenhouse (14 h photoperiod, 25/20 °C day/night temperature, 70% relative humidity). Each pot was fertilized with 200 mL of Yoshida nutrient solution[36] per week. The grains were harvested 135 days after transplanting and dried at 80 °C. Brown rice, obtained by removing the rice hull, was powdered, sieved to pass mesh number 40 (W. S. Tyler, USA), and stored at 4 °C until further use. The description of seedling preparation is presented in the Supporting Information (SI).

Cerium and Macro- and Micronutrient Concentrations in Rice Seeds. Rice grains (100 mg) were microwave digested (CEM Mars$_X$ Mathews, NC, USA) using a mixture of plasma pure HNO$_3$ and H$_2$O$_2$ (1:4).[37] Elemental analysis was performed using inductively coupled plasma−optical emission spectroscopy, whereas Ce quantification was achieved using ICP−mass spectroscopy, following a previously described method.[37] Blank, spikes, and standard reference material (NIST-SRF 1570a) were used to validate the digestion and analytical method.

Analysis of Antioxidant Property. The extract for the analysis of antioxidant property was prepared on the basis of Adom and Liu.[38] The total phenolic and flavonoid were estimated according to the methods of Dewanto et al.[39] and Jia et al.,[40] respectively. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis(3-ethylene-benzothiozoline-6-sulfonic acid) (ABTS) radical cation scavenging abilities were determined on the basis of Williams et al.[41] and Arts et al.,[42] respectively. The description of methods is shown in the SI.

Sugars and Starch Analysis. Total and reducing sugars were extracted following a previously described method.[43] Starch and total sugar were quantified according to the method of Dubois et al.,[44] whereas reducing sugar content was determined on the basis of the method of Nelson-Somogyi.[45] The nonreducing sugar content was obtained from the difference between total and reducing sugars. The methods are shown in the SI.

Protein Analysis. The protein content of rice grains was fractionated according to the method of Chen and Bushuk.[46] Protein from rice grain (500 mg) was extracted sequentially with 8 mL each of water, 0.5 M NaCl, 70% ethanol, and 0.05 M acetic acid and labeled as albumin, globulin, prolamin, and glutelin fractions, respectively. The protein content was quantified by using the Bradford method.[47]

Fatty Acid Analysis. Fatty acids in rice grains were esterified and extracted according to the method of Browse et al.[48] and analyzed by GC-MS using a Gerstel Twister desorption unit (Gerstel, Inc., Baltimore, MD, USA). A series of working calibration standards with concentrations of 0.5, 1, 10, 50, and 100 μg L^{-1} were applied for retention time identification and response curve generation. A total of three replicates were used. The SI presents the detailed description for GC-MS analysis.

FT-IR/ATR Analysis. The spectra of powdered rice were collected using an FT-IR/ATR spectrometer 100 (Perkin-Elmer, Shelton, CT, USA) applying these settings: 2 cm^{-1} resolution, 4 number of scans, and air as background. The second-derivative spectrum was calculated using Spectrum software (version 6.0.2.0025, Perkin-Elmer). A total of three replicates were used for the analysis.

Statistical Analysis. This study investigated two factors and their interaction. nCeO$_2$ treatment (A) was the first factor, which is composed of control and 500 mg nCeO$_2$ kg^{-1} referred to as untreated and treated, respectively. Rice variety (V) is another factor, which includes high-, medium-, and low-amylose varieties (HA, MA, and LA, respectively). Data were analyzed using SAS statistical package version 9.3 (SAS Institute, Cary, NC, USA). A two-way ANOVA using the General Linear Model was performed with the significance of the varietal means tested with Tukey's honestly significant difference test based on a probability of $p \leq 0.05$ except when otherwise stated. General treatment means were compared using least significant differences. All values are on a dry weight (d wt) basis.

RESULTS AND DISCUSSION

Treatment Effects on Parameters Investigated. The ANOVA of parameters measured is presented in Tables S1 and S2 of the Supporting Information. Results showed that the nCeO$_2$ factor (A) significantly affected the Ce, K, Ca, S, and Fe concentrations and all antioxidant properties, except flavonoid content. nCeO$_2$ also affected the albumin, prolamin, and starch contents. ANOVA also revealed a large varietal (V) effect on most of the parameters, except for Cu concentration, DPPH, and total and nonreducing sugars. On the other hand, the interaction between A and V was significant for all protein fractions, lauric and valeric acids, phenolic content, and Ce, Al, Fe, K, Mn, and Zn concentrations. These findings are in agreement with reports showing variety as an important factor in rice grain quality.[11,32,49]

Cerium Concentration in Rice Grains. The accumulation of ENPs such as nCeO$_2$, nC$_{60}$(OH)$_{20}$, and nC$_{70}$ in soybean pods,[6] bitter melon fruits,[31] and rice grains,[50] respectively, has been documented. Ce accumulation from the nCeO$_2$ treatment in rice grains is presented in Table 1. Data showed that Ce

Table 1. Cerium Concentration (Micrograms per Kilogram Dry Weight) in Rice Grains Cultivated in Soil Treated or Not with 500 mg of nCeO$_2$ kg^{-1}[a]

rice variety	untreated	500 mg nCeO$_2$ kg^{-1}
high amylose	115 ± 48a	224 ± 17b^{ns}
medium amylose	156 ± 10a	1912 ± 383a***
low amylose	169 ± 29a	1853 ± 460a**
mean	147 ± 21	1330 ± 341***

[a] Values are means ± SE, $n = 4$. Between rice varieties, means with the same letter are not significantly different at Tukey's test ($p \leq 0.10$). Between nCeO$_2$ treatments, ns is not significant at $p \leq 0.05$; **, and *** indicate significance at $p \leq 0.01$, and $p \leq 0.001$, respectively.

concentration in the HA variety did not change with $n\mathrm{CeO_2}$ treatment. On the other hand, Ce concentrations in treated MA and LA varieties were elevated by 1126 and 996%, respectively, compared to control. Comparison between $n\mathrm{CeO_2}$-treated plants showed that MA and LA accumulated more Ce than the HA variety. In the treatment means, the treated plants yielded Ce content that is remarkably higher by 805% than the untreated plants, indicating that $n\mathrm{CeO_2}$ treatment is a significant factor for Ce accumulation in rice grains. Similarly, $n\mathrm{CeO_2}$ treatment greatly increased Ce concentration in soybean pods and tomato (*Solanum lycopersicum* L.) fruits.[5,51] Further studies should be performed to determine the speciation of Ce in the grains.

Nutrient Contents of Rice Grains. Dietary minerals such as micro- (Fe, Zn, Se, and Cu) and macronutrients (Ca and Mg) are primarily obtained in diets that are reliant on grains.[11] These minerals are often lacking in human diets that biofortification is undertaken to boost their concentrations in food crops.[52] Table 2 shows the nutrient profile in grains harvested from $n\mathrm{CeO_2}$-treated and untreated soils. As seen in the table, element concentrations in grains of the three rice varieties were different from each other except for Al, Cu, Mn, and Na in the untreated and Cu, Na, and Zn in the treated samples. Comparison between treatments showed that relative to the control, S concentration in treated HA was lower by 6.2%, whereas Fe concentration in treated LA was significantly reduced (∼69% lower). For the MA variety, K, Na, Fe, and Al concentrations in the treated plants were markedly higher than in untreated plants (20.7, 7.6, 425, and 174.2% higher, respectively), whereas S concentration in treated plants significantly dropped by 7.5% compared with untreated samples. In the case of treatment means, K and Ca concentrations in treated were higher than the control by 8.8 and 25.5%, respectively, whereas S and Fe concentrations in treated were lower than the untreated samples by 5.9 and 30.4%, respectively.

The increase in K and Ca in grains, although negatively affecting eating quality, is beneficial for human nutrition.[53] On the other hand, the decrease in Fe could exacerbate the globally prevalent problem of Fe deficiency for those whose diets are primarily based on rice,[54] whereas reduction in S could affect protein and glutathione synthesis and the antioxidant capacity of grains.[55] The effects of $n\mathrm{CeO_2}$ on K, Ca, and Fe were in agreement with those observed in rice shoots treated with $\mathrm{Ce^{3+}}$ (0.5 and 1.0 mM $\mathrm{Ce^{3+}}$), wherein the uptake of K and Ca increased while the uptake of Fe decreased.[56] On the contrary, *n*Pd decreased the Ca concentration in kiwifruit (*Actinidia deliciosa*) pollen.[17] On the other hand, carbon nanotubes did not change the Ca accumulation in *Spartina alterniflora*,[18] and $n\mathrm{TiO_2}$ did not affect the K concentration in *Ulmus elongata*.[19]

The Na/K, Na/Ca, and Mg/K ratios are indicators of stress and quality in plants. These ratios were measured and are displayed in Table S3 in the SI. In the treatment means, Na/K was not affected by $n\mathrm{CeO_2}$, whereas the Na/Ca ratio notably decreased in the treated (0.586) relative to the untreated samples (0.742), suggesting an increased competitive inhibition between the uptake of Na and that of Ca.[18] It has been reported that the accumulation of K and Ca and reduction in Na/Ca ratio by carbon nanotubes mitigated the harmful effects of Cd in *S. alterniflora*.[18] In the present study, the data also revealed that the Mg/K ratio, an indicator of eating quality of rice,[53] was greatly reduced in the treated (0.391) compared to the untreated (0.435) samples. The decrease in Mg/K ratio with concomitant increase in K and Ca contents indicates poor

Table 2. Macronutrient Concentrations (Milligrams per Kilogram Dry Weight) in Rice Grains Cultivated in Control Soil and Soil Treated or not with $n\mathrm{CeO_2}$ at 500 mg $\mathrm{kg^{-1}}$ Soil[a]

	P	S	K	Mg	Ca	Na	Zn	Fe	Cu	Mn	Al
					Untreated						
HA	66.1 ± 0.8a	161.8 ± 1.7b	413.6 ± 5.5b	163.5 ± 1.2b	6.9 ± 1.7b	9.4 ± 0.1a	2.3 ± 0.3b	1.5 ± 0.1b	2.6 ± 0.2a	0.78 ± 0.14a	8.9 ± 3.1a
MA	55.7 ± 1.7b	183.4 ± 3.4a	316.2 ± 7.7c	160.1 ± 5.1b	18.0 ± 1.5a	10.5 ± 0.2a	4.4 ± 0.2a	0.4 ± 0.1c	2.8 ± 0.1a	0.90 ± 0.02a	6.2 ± 0.8a
LA	69.0 ± 0.2b	164.6 ± 2.7b	438.9 ± 1.7a	176.8 ± 1.1a	21.2 ± 0.6a	9.7 ± 0.4a	5.2 ± 0.3a	4.9 ± 0.2a	2.7 ± 0.1a	0.79 ± 0.05a	20.4 ± 5.6a
mean	63.6 ± 1.8	169.9 ± 3.2	389.5 ± 16.2	166.8 ± 2.7	15.3 ± 2.0	9.9 ± 0.2	4.0 ± 0.4	2.3 ± 0.6	2.8 ± 0.1	0.83 ± 0.05	11.8 ± 2.7
					500 mg $n\mathrm{CeO_2}$ $\mathrm{kg^{-1}}$						
HA	65.2 ± 0.2a^{ns}	151.7 ± 3.6b*	424.9 ± 5.1ab^{ns}	163.6 ± 1.8b^{ns}	11.0 ± 12a^{ns}	10.2 ± 0.7a^{ns}	3.2 ± 0.4a^{ns}	1.1 ± 0.3b^{ns}	2.9 ± 0.2a^{ns}	0.54 ± 0.03b*	8.8 ± 1.4b^{ns}
MA	57.7 ± 2.8b^{ns}	169.6 ± 4.2a*	381.7 ± 25.2b*	153.4 ± 4.6b^{ns}	22.2 ± 3.4a^{ns}	11.3 ± 0.2a*	3.1 ± 0.7a^{ns}	2.1 ± 0.1a*	2.5 ± 0.1a^{ns}	0.93 ± 0.02a^{ns}	17.0 ± 1.2a*
LA	69.7 ± 0.8a^{ns}	158.5 ± 1.7ab^{ns}	464.5 ± 11.6a^{ns}	178.2 ± 2.8a^{ns}	24.5 ± 1.4b^{ns}	10.2 ± 0.5a^{ns}	4.8 ± 0.3a^{ns}	1.5 ± 0.1ab*	2.8 ± 0.1a^{ns}	0.86 ± 0.02a^{ns}	12.3 ± 0.1b^{ns}
mean	64.2 ± 1.7^{ns}	159.9 ± 2.8**	423.7 ± 13.3**	165.1 ± 3.5^{ns}	19.2 ± 2.1*	10.5 ± 0.3^{ns}	3.7 ± 0.4^{ns}	1.6 ± 0.2**	2.7 ± 0.1^{ns}	0.77 ± 0.05^{ns}	12.7 ± 1.2^{ns}

[a] Values are means ± SE, $n = 4$. HA, MA, LA = high-, medium-, and low-amylose variety, respectively. Between rice varieties, means with the same letter are not significantly different at Tukey's test ($p \leq 0.05$). Between $n\mathrm{CeO_2}$ treatments, ns is not significant at $p \leq 0.05$; *, **, and *** indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

eating quality,[53] signifying that $n\mathrm{CeO_2}$ caused deterioration in the eating quality of rice.

Antioxidant Property of Rice Grains. Rice grains contain phenolic compounds and possess electron or radical scavenging abilities that help reduce the risk of chronic diseases.[38,57] The influence of $n\mathrm{CeO_2}$ on the phenolic content and radical scavenging ability of rice grains is presented in Table 3. In the untreated samples, phenolic and flavonoid concentrations were highest in HA and LA, respectively, whereas ABTS was lowest in HA. A similar trend was observed in treated grains. DPPH in both treated and untreated samples remained the same. Comparison between $n\mathrm{CeO_2}$ treatments revealed that there were no changes in the antioxidant activities in MA, except for flavonoid concentration in treated samples, which increased by 12.5% relative to the control. On the other hand, the $n\mathrm{CeO_2}$-treated HA and LA grains displayed marked reduction in phenolic contents (28.2 and 32.9%, respectively) and DPPH scavenging ability (42.8 and 34.3%, respectively) compared to the untreated ones. In the case of treatment means, phenolic content, DPPH, and ABTS were significantly reduced in treated samples by 24.1, 27.9, and 12.8%, respectively, compared with untreated samples. Moreover, the data showed that HA and LA varieties were more sensitive to changes in antioxidant value compared to MA.

The results are in agreement with the inverse relationship found between magnetic nanoparticle concentration and antioxidant activity in tobacco BY-2 cells but opposite to the direct relationship observed between nAg concentration and antioxidant activity in castor seedlings reported in the literature.[20,21] The decreases in phenolic content and radical scavenging ability indicate the antioxidative activity of plants under metal stress because it plays a protective role in metal chelation and reactive oxygen scavenging.[58,59] $n\mathrm{CeO_2}$ exhibits antioxidant-like property and has been found to influence the antioxidative enzymes in corn and rice,[7,60] but there is a dearth of information regarding its effects on the phenolic compounds and radical scavenging ability in plants. The current findings indicate that $n\mathrm{CeO_2}$ cause a negative effect on the antioxidant capacity of rice grains, which could translate to reduced nutritional value of rice grain.

Protein Content in Protein Fractions of Rice Grains. The effects of $n\mathrm{CeO_2}$ on the concentrations of albumin, globulin, prolamin, and glutelin fractions of rice grain proteins are presented in Table 3. In control treatment, MA grains had the highest concentrations in albumin, globulin, and prolamin, whereas LA had the lowest. The concentration of glutelin did not vary between rice varieties. For the $n\mathrm{CeO_2}$-treated plants, globulin did not change among the rice varieties, whereas glutelin was not detected, suggesting these protein fractions are most sensitive to $n\mathrm{CeO_2}$ toxicity. Comparison between treatments showed that the protein contents, except for glutelin, did not change in HA. On the other hand, the protein contents in LA increased in treated (2.79−8.88 mg g^{-1}) compared with untreated (2.41−7.72 mg g^{-1}) plants, and those of MA, except for albumin, decreased in the treated (4.32−8.51 mg g^{-1}) relative to the untreated (6.84−9.71 mg g^{-1}) plants. Comparison between treatment means revealed that $n\mathrm{CeO_2}$ affected the protein contents in all fractions except globulin. The $n\mathrm{CeO_2}$ treatment increased the protein content in albumin by 7.3%, but greatly decreased that in prolamin by 17.4%, compared to the control. In general, $n\mathrm{CeO_2}$ decreased the protein content by 17% in MA and increased it by 19% in LA relative to the untreated but did not change in HA, indicating

Table 3. Antioxidant Property and Nutritional Content of Rice Grains Cultivated in Soil Treated or not with $n\mathrm{CeO_2}$ at 500 mg kg^{-1} Soil[a]

	antioxidant property				protein content (mg g^{-1} d wt)				starch and sugar content (mg g^{-1} d wt)			
rice variety	phenolic content (μg GAE g^{-1})	flavonoid content (μg catechin g^{-1})	DPPH scavenging (%)	ABTS scavenging (%)	albumin	globulin	prolamin	glutelin	starch	total sugar	reducing sugar	nonreducing sugar
					Untreated							
HA	959 ± 7a	99 ± 5b	16.6 ± 1.9a	3.1 ± 0.2b	5.45 ± 0.12ab	9.87 ± 0.18a	3.90 ± 0.09b	0.27 ± 0.03 a	731 ± 17a	8.00 ± 0.59a	3.10 ± 0.13a	4.90 ± 0.46a
MA	464 ± 5b	88 ± 6b	10.6 ± 2.9a	4.3 ± 0.1a	5.95 ± 0.05a	9.71 ± 0.42a	6.84 ± 0.11a	0.21 ± 0.04 a	663 ± 24a	8.86 ± 0.27a	2.58 ± 0.16ab	6.28 ± 0.22a
LA	420 ± 20b	116 ± 5a	16.9 ± 0.7a	4.4 ± 0.1a	5.11 ± 0.21b	7.72 ± 0.20b	2.41 ± 0.10c	0.31 ± 0.03 a	723 ± 7a	8.26 ± 0.80a	2.17 ± 0.17b	6.09 ± 0.82a
mean	628 ± 80	102 ± 4	14.7 ± 1.4	3.9 ± 0.2	5.50 ± 0.13	9.10 ± 0.33	4.38 ± 0.56	0.27 ± 0.02	706 ± 13	8.37 ± 0.33	2.62 ± 0.14	5.76 ± 0.34
					500 mg $n\mathrm{CeO_2}$ kg^{-1}							
HA	689 ± 29a***	93 ± 2b ^{ns}	9.5 ± 0.3a**	2.7 ± 0.5b^{ns}	5.08 ± 0.11b^{ns}	9.09 ± 0.48a^{ns}	3.76 ± 0.02b^{ns}	nd	664 ± 10a*	9.25 ± 0.52a^{ns}	2.81 ± 0.17a^{ns}	6.44 ± 0.38a^{ns}
MA	453 ± 31b^{ns}	99 ± 4b*	11.1 ± 0.5a^{ns}	3.8 ± 0.1ab^{ns}	5.75 ± 0.25b^{ns}	8.51 ± 0.10a**	4.32 ± 0.03a**	nd	625 ± 24a^{ns}	9.00 ± 0.50a^{ns}	2.48 ± 0.10a^{ns}	6.52 ± 0.60a^{ns}
LA	282 ± 6c***	115 ± 5a^{ns}	11.1 ± 1.0a*	3.8 ± 0.1a^{ns}	6.87 ± 0.17 a***	8.87 ± 0.10a*	2.79 ± 0.04 c***	nd	666 ± 5a*	7.49 ± 0.74a^{ns}	2.34 ± 0.08a^{ns}	5.14 ± 0.69a^{ns}
mean	477 ± 57***	103 ± 4^{ns}	10.6 ± 0.4**	3.4 ± 0.2*	5.90 ± 0.24**	8.82 ± 0.17 ^{ns}	3.62 ± 0.19***		651 ± 10 ***	8.58 ± 0.39^{ns}	2.54 ± 0.09 ^{ns}	6.03 ± 0.35 ^{ns}

[a] Values are means ± SE, $n = 4$. HA, MA, LA = high-, medium-, and low-amylose variety, respectively. GAE = gallic acid equivalent. Between rice varieties, means with the same letter are not significantly different at Tukey's test ($p \leq 0.05$). Between $n\mathrm{CeO_2}$ treatments, ns is not significant at $p \leq 0.05$; *, **, and *** indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Table 4. Fatty Acid Contents (Micrograms per Kilogram Dry Weight) of Rice Grains Cultivated in Soil Treated or not with $n\mathrm{CeO_2}$ at 500 mg $\mathrm{kg^{-1}}$ Soil[a]

rice variety	lauric acid (C12)	myristic acid (C14)	valeric acid (C15)	palmitic acid (C16)	oleic acid (C18:1)	linoleic acid (C18:2)	linolenic acid (C18:3)	total
				Untreated				
HA	275 ± 18b	317 ± 93a	333 ± 6b	3701 ± 756a	2127 ± 504a	4944 ± 1289a	1192 ± 456a	12888 ± 3071a
MA	424 ± 21a	281 ± 40a	527 ± 15a	5054 ± 557a	3156 ± 463a	6574 ± 769a	1164 ± 89a	17181 ± 1882a
LA	260 ± 10b	229 ± 41a	329 ± 2b	3368 ± 399a	1735 ± 292a	4237 ± 714a	686 ± 77a	10846 ± 1488a
mean	320 ± 20	276 ± 35	397 ± 23	4041 ± 365	2340 ± 275	5252 ± 572	1014 ± 158	13638 ± 1378
				500 mg $n\mathrm{CeO_2}$ $\mathrm{kg^{-1}}$				
HA	283 ± 16a$^{\mathrm{ns}}$	315 ± 49a$^{\mathrm{ns}}$	324 ± 4b$^{\mathrm{ns}}$	3811 ± 484a$^{\mathrm{ns}}$	2081 ± 290a$^{\mathrm{ns}}$	4801 ± 677a$^{\mathrm{ns}}$	867 ± 93a$^{\mathrm{ns}}$	12483 ± 1571a$^{\mathrm{ns}}$
MA	246 ± 6a***	174 ± 24b$^{\mathrm{ns}}$	310 ± 5b***	3378 ± 508a*	1810 ± 347a*	4275 ± 962a$^{\mathrm{ns}}$	705 ± 107a$^{\mathrm{ns}}$	10898 ± 1911a*
LA	282 ± 9a$^{\mathrm{ns}}$	285 ± 18ab$^{\mathrm{ns}}$	343 ± 2a$^{\mathrm{ns}}$	4113 ± 434a$^{\mathrm{ns}}$	2320 ± 318a$^{\mathrm{ns}}$	5669 ± 730a$^{\mathrm{ns}}$	857 ± 82a$^{\mathrm{ns}}$	13868 ± 1552a$^{\mathrm{ns}}$
mean	270 ± 7***	258 ± 23$^{\mathrm{ns}}$	326 ± 4***	3768 ± 268$^{\mathrm{ns}}$	2070 ± 180$^{\mathrm{ns}}$	4915 ± 455$^{\mathrm{ns}}$	810 ± 54$^{\mathrm{ns}}$	12417 ± 961$^{\mathrm{ns}}$

[a] Values are means ± SE, $n = 4$. HA, MA, LA = high-, medium-, and low-amylose variety, respectively. Between rice varieties, means with the same letter are not significantly different at Tukey's test ($p \leq 0.05$). Between $n\mathrm{CeO_2}$ treatments, ns is not significant at $p \leq 0.05$; *, **, and *** indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

that $n\mathrm{CeO_2}$ is detrimental to MA, beneficial to LA, and insignificant to HA.

Studies have demonstrated that ENPs induce modifications on the protein levels in plants at early seedling stage.[18,20,22−25] However, its mechanism is not yet clear. Further studies are needed to elucidate the mechanism by which $n\mathrm{CeO_2}$ alter the protein content in the grain. It is possible that $n\mathrm{CeO_2}$ affect the gene expression for protein synthesis during grain development similar to those observed in rice under drought conditions.[16]

Starch and Sugar Contents of Rice Grains. Rice is considered a valuable crop for human nutrition because it contains more energy to support more people per unit of land.[10] Table 3 presents the effects of $n\mathrm{CeO_2}$ on the starch and sugar contents of rice grains. Results showed that the $n\mathrm{CeO_2}$ treatment did not change the sugar contents but affected the starch concentration. HA and LA grains yielded starch contents in the treated sample that were lower than the untreated by 9.2 and 7.9%, respectively. Similarly, treatment means showed that $n\mathrm{CeO_2}$ significantly decreased the starch concentration by 7.8% compared with the untreated. In general, the results demonstrated that $n\mathrm{CeO_2}$ greatly reduced the starch concentration in HA and LA varieties.

The modifications in starch and sugar contents of ENP-treated plants as indicators of toxicity have been reported.[18,22,24,26,27] In the case of studies in mature plants, foliar applications of nAu increased the amount of total and reducing sugars in the harvested seeds from *Brassica juncea* L.,[28] whereas spraying of nAg resulted in high total soluble solids in fruits of cucumber.[29] The mechanism of starch modification by $n\mathrm{CeO_2}$ in rice grains has yet to be elucidated. Related studies revealed that Ni disrupted the conversion of starch into sucrose, resulting in reduced carbohydrate levels in rice roots,[61] whereas Cd triggered dramatic perturbations in starch and sugar syntheses in rice roots and shoots.[43] Another study revealed that drought effected the carbohydrate metabolism involved in starch biosynthesis in developing rice grain.[16]

Fatty Acid Content in Rice Grains. Rice provides 3% of dietary fat in rice-consuming countries.[62] The effects of $n\mathrm{CeO_2}$ on the FA profile of rice grains are presented in Table 4. In untreated grains, the MA variety displayed the highest amounts of lauric and valeric acids. In the $n\mathrm{CeO_2}$-treated plants, the HA variety yielded the highest concentration of myristic acid, whereas LA had the highest amount of valeric acid. Comparison between treatments revealed that concentrations of FA in HA and LA varieties did not change. However, compared to control, the $n\mathrm{CeO_2}$-treated MA grains had a significant decrease in lauric, valeric, palmitic, and oleic acids (41.9, 41.2, 33.2, and 42.7%, respectively) as well as total FA (36.6%). Comparison between treatment means showed that lauric and valeric acids were most sensitive to $n\mathrm{CeO_2}$ treatments (Table 4). However, the most abundant FAs (palmitic, oleic, and linoleic acids), which comprise 95% of the total FA in rice grains, were not affected.

Recent reports showed that nS and nZnO altered the lipid content in bean and corn tissues,[25] whereas nAu increased the oil content of seeds harvested from mustard.[28] A survey of the current literature reveals that ENPs promote membrane damage, which most likely affects the FA profile in plants, similar to those observed under heavy metal stress.[63] The current understanding on the membrane damage in plants due to ENPs exposure should be explored to understand their effects on FA contents in plants.

FTIR Analysis of Rice Grains. Recently, FTIR was employed in determining changes in the chemical makeup of mustard and rice tissues exposed to multiwalled carbon nanotubes and nAg, respectively,[27,64] *Ulmus elongata* seedlings sprayed with $n\mathrm{TiO_2}$[19] and tomato (*Lycopersicum esculentum* L.) seeds germinated in nAg.[65] However, FTIR analysis of ENP-treated grains has not yet been reported. Figure 1 displays the FTIR spectra of rice grains harvested from the $n\mathrm{CeO_2}$-treated plants. All rice varieties showed differences between the spectra of untreated and treated rice grains. Both the carbohydrate and amide regions in all varieties showed changes in the IR intensities; however, a more dramatic modification was observed in the lipid regions of both HA and MA. It is interesting to note that $n\mathrm{CeO_2}$ caused changes in the IR intensity similar to those reported in $n\mathrm{TiO_2}$-sprayed *U. elongata* leaves[19] and nAg-treated tomato seeds,[65] but no shifting of bands similar to those observed in the roots of nAg-treated rice[64] was found.

Pearson's Correlations. Some parameters showed significant Pearson's correlations with Ce as shown in Table 5. As seen in this table, Ce was positively correlated with Ca, Na, and albumin and negatively correlated to phenolic and starch. The positive correlation between Ce and Ca concentrations is in agreement with papers showing increased absorption of Ca in rice and *Arabidopsis thaliana* treated with $\mathrm{Ce^{3+}}$.[56,67] A high Na concentration could be toxic in rice grain; however, the simultaneous increase in Ca, which was statistically higher in

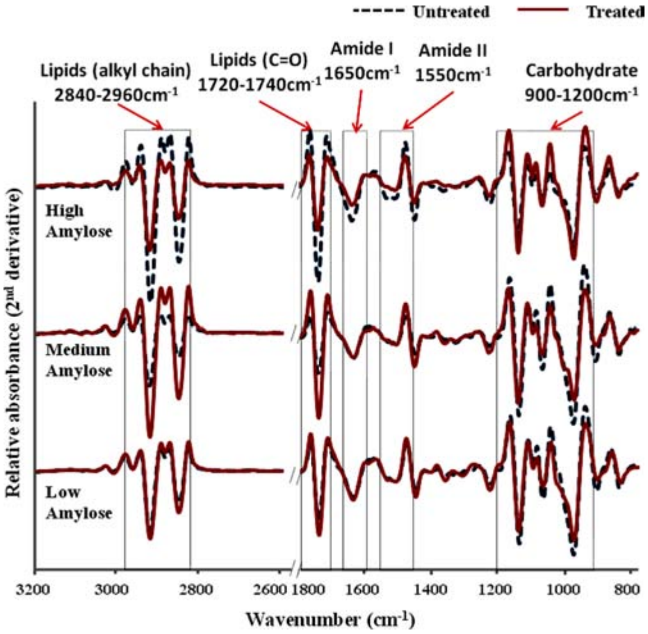

Figure 1. FTIR spectra of rice grain powder of different rice varieties harvested from plants cultivated in soil treated or not with $n\mathrm{CeO_2}$ at 500 mg $\mathrm{kg^{-1}}$. Assignment of spectra region was adapted from Dokken et al.[66]

Table 5. Significant Pearson's Correlations Between Measured Parameters and Ce Concentration in Grains of Rice Plants Grown in Soil Treated or Not with $n\mathrm{CeO_2}$ at 500 mg $\mathrm{kg^{-1}}$ Soil[a]

Ca	0.56025*
Na	0.48145*
phenolic content	−0.51249*
albumin	0.64734**
starch	−0.59486**

[a] * and ** indicate significance at $p \leq 0.05$ and $p \leq 0.01$, respectively.

treated compared with untreated grains (Table 2), indicates that Ca could have mitigated the detrimental effect of Na.[68] In addition, higher accumulation of Ce in albumin, compared to other protein fractions, in rice tissues treated with Ce has also been reported.[69] The inverse relationship between Ce and phenolic concentrations is in agreement with the generally observed role of phenolic compounds in sequestering heavy metals in plants.[58] It is possible that the reduced phenolic concentration obtained in the present study could be due to the increased Ce content in the grains (Table 3). The negative correlation between Ce and starch concentrations suggests that $n\mathrm{CeO_2}$ will have a negative impact on the nutritional value of rice, because, in dry weight, this contains 90% starch and provides 27% of dietary energy supply in more than 33 developing countries.[62]

In summary, the findings demonstrate that $n\mathrm{CeO_2}$ modified the nutritional value of rice, which may have a long-term negative effect in food quality. This study provides the first proof that $n\mathrm{CeO_2}$ can have significant impacts on the nutritive value of rice.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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