Environmental Impact and Nutritional Improvement of Elevated CO_2 Treatment: A Case Study of Spinach Production

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Abstract: The agriculture sector is known to be the one of the major contributors to global greenhouse gas (GHG) emissions. At the same time, global climate changes have affected the agriculture sector. In order to strengthen the sustainable development of agriculture, it is important to promote environmentally friendly farming and simultaneously increase the economic value. To improve the productivity of agriculture, technical advancements have occurred. Among those, we have focused on CO_2 treatment in cultivation. We aimed to clarify the effectiveness of the elevated CO_2 treatment of spinach based on GHG emission and the economic value using the eco-efficiency score. We assumed that nutrition could represent the value of the vegetable. We measured weights, vitamin C, and CO_2 emissions of elevated CO_2 treatment and conventional production. We used life cycle assessment (LCA) to estimate CO_2 emissions. CO_2 emissions of a 100-g bouquet of spinach were estimated from agricultural inputs, farming, transport, and distribution center processes at a model spinach farm in Japan. CO_2 emission of elevated CO_2 treatment was 29.0 g-CO_2, and was 49.0 g-CO_2 for conventional production. The net weight of a bouquet of elevated CO_2-treated spinach was 1.69-fold greater than that of conventional production. Vitamin C per 100 g spinach produced via elevated CO_2 treatment was 15.1 mg, and that of conventional production was 13.5 mg on average. Finally, based on the above results, we assessed the eco-efficiency scores of the elevated CO_2 treatment and conventional production of spinach, enabling integration of the nutritional value and the environmental impact. The score showed that elevated CO_2 treatment (0.76) was 2.9-fold more efficient than conventional production (0.26). This study suggested that elevated CO_2 treatment could enhance growth and nutritional value of spinach, and further contribute to CO_2 reduction.

Keywords: LCA; elevated CO_2 treatment; spinach; vitamin C; eco-efficiency

1. Introduction

Our concerns about global warming have stimulated the development of environmental assessment of agriculture sectors using life cycle assessment (LCA) analysis. LCA has been used since the end of the 1990s to assess the environmental impact of single crop cultivation, and has shifted to various agriculture sectors: animal production, horticulture, organic farming, etc. [1–3]. In Europe, it is promoted to certify environmentally-assessed products, and EPD (Environmental Product Declaration) was introduced to reduce the environmental burden of production. EPD is an independently verified and registered document that communicates comparable information about the life-cycle environmental impact of products [4,5]. It contains more than 700 products' documents.

In order to strengthen the sustainability of agriculture, it is very important to increase both the prevalence of environmentally friendly farming the economic value of the sector. Towards this concern,

ISO 14045 determined the eco-efficiency protocol in 2012. Eco-efficiency is meant to support sustainable development. It is a quantitative management tool which enables the consideration of the life cycle environmental impacts of an agricultural system alongside its product system value to a stakeholder [6–8]. The value of a vegetable could be enhanced by the production of the product in a manner that enlarges values such as productivity, nutrition, taste, etc. Productivity and nutrition are especially important in Japan under the recent shrinking agriculture sector and changing dietary patterns. Agriculture in Japan has been resistant to change, where small-plot holdings still dominate, the average farmer is over 66 years old, and the sector's contribution to the economy has fallen by 25 percent since its peak in 1984. To address these factors, technological advances have been introduced to increase the productivity. For example, elevated CO_2 treatment—in which CO_2 is input higher than ambient (400 ppm)—has been recorded to yield three-to-four-fold production quantity compared to conventional farming. CO_2 is a raw material for photosynthesis, and its application to plants has been proven to improve productivity [9–15]. It has been introduced as a standard technique in protected horticulture, especially in the Netherlands. It is expected that CO_2—one of the major greenhouse gases—could become a useful resource in agriculture. In Japan, it has been tested in a few agricultural sectors: tomato, strawberry, rose, etc. [16–18]. However, it is not yet broadly applied at farms, but in 1448 ha of protected horticulture facilities, which accounts about 3.1% of the total in 2015 [19].

Regarding the changing dietary habits of Japanese people, we assumed that nutrition could be a value to attract consumers, followed by increasing economic value. The consumption of meat and excessive fats has been expanding, and the dietary eccentricity has advanced rapidly. Poor diet is more prone to disease, infection, fatigue, and poor performance. Hence, health-conscious consumers are driving demand for strains of vegetables with enhanced nutritional qualities. So-called "functional vegetables" in Japan—for example, broccoli containing three times the amount of antidotal and antioxidant elements, carrots containing a balanced content of the antioxidants lycopene and carotene, etc.—command higher prices than ordinary. Farmers are eager to comply with the above value-added vegetables.

Concerned with environmental issues in the agriculture sector, the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan formulated a "Competitive Agriculture" policy, promoting the use of renewable energy on farms [20]. It was deemed that agriculture and fishing villages have plentiful abundance of renewable energy resources such as biomass, sunlight, and water. Our interest was the CO_2 waste exhausted from some renewable energy plants. We assumed that waste CO_2 could be a source of elevated CO_2 treatment.

From the above backgrounds, we aimed to prove the effectiveness of elevated CO_2 treatment on leafy vegetables in a protected horticulture system, where it was assumed that CO_2 would be supplied from the waste of a renewable energy plant. We estimated the eco-efficiency, which was determined per ISO 14045 [21]. We integrated the environmental impact and the nutritional quality. We chose spinach as a model leafy vegetable. First, we performed a growth experiment of spinach under different CO_2 concentrations: elevated CO_2 treatment (eCO$_2$ system) and conventional production (Conv system). We measured the weights and vitamin C contents of those two systems, respectively. Secondly, we conducted LCA analysis to estimate CO_2 emissions of those systems, respectively. Finally, we calculated eco-efficiency score using vitamin C contents and CO_2 emissions. The scores of those systems were compared.

2. Materials and Method

2.1. Incubator-Scale Cultivation of Spinacia oleracea

We performed the growth experiment using *Spinacia oleracea* as model leafy vegetable. Germinated *Spinacia oleracea* pots were moved into a plant incubator (Biotorn, NKsystem Co., Ltd. Tokyo, Japan) which is able to automatically control the environment of the plant. It can control the temperature, moisture, and lighting. We prepared the two different CO_2 concentration systems:

ambient conventional system (Conv system, 400 ppm), and elevated CO_2 treatment (eCO_2 system, 1000 ppm). We cultivated each system three times including four samples, respectively. They were produced for 20 days. CO_2 was continuously flowed in. Incubator was set to 16 h lighting time, 22 °C temperature, 50% humidity, and 60% illumination (Table 1). Culture soil was mildly acidic with 170 mg/L nitrogen (N), 140 mg/L phosphorous (P), and 120 mg/L potassium (K). These conditions were kept throughout the growth experiment.

2.2. Measurement of Weight and Vitamin C

A bouquet of spinach was washed and weighted using an electronic balance (A&D Co., Ltd., Tokyo, Japan) after 20 days of cultivation. Vitamin C was measured according to the manufacturer's procedure using a vitamin C assay kit (Shima Laboratories Co., Ltd., Tokyo, Japan). Briefly, we used a standard solution of ascorbic acid to clarify a calibration curve, showing the relationship between absorbance and vitamin C concentration due to the difference in dilution ratio. Additionally, 100 g of edible portion of spinach was homogenized with a five-fold amount of a 5.4% metaphosphoric acid aqueous solution, and centrifuged at 15,000 rpm, 10 min, 4 °C. Supernatant was measured using a spectrophotometer. The measurement was performed three times for each system. Vitamin C concentration was calculated using a calibration curve.

2.3. Life Cycle Assessment (LCA)

LCA in this study was conducted by inventory analysis. CO_2 emissions of the Conv system were calculated at the model protected horticulture system of spinach in Asakura City, Fukuoka prefecture. The model farm ranged 3 a in area and produced 720 kg spinach for 24 days.

The functional unit was 100 g of harvested spinach. The system boundary of the Conv system includes agricultural inputs, farming, transport, and distribution center processes, as shown in Figure 1. That of the eCO_2 system includes the same as the Conv system and CO_2 production. However, CO_2 emissions of CO_2 production were ignored in this study, because we set that CO_2 was supplied from the waste CO_2 of renewable energy plants.
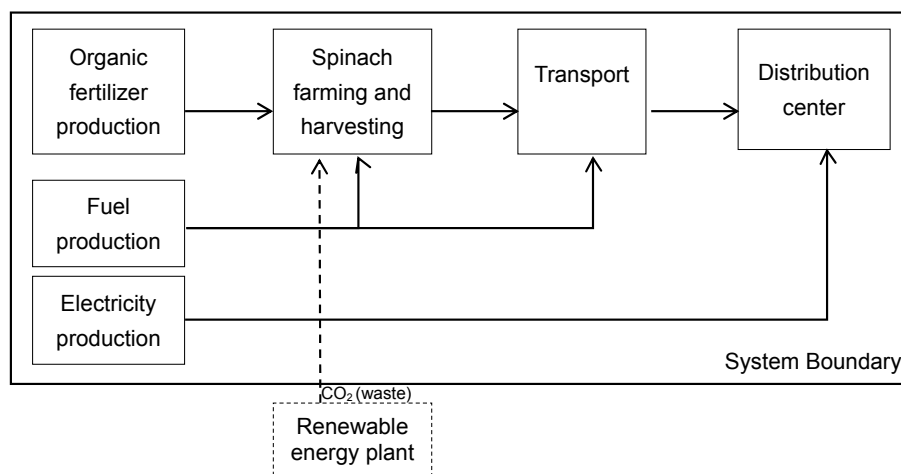
Figure 1. System boundary of life cycle assessment (LCA) approach at the model spinach farm. Because it was assumed to be from the waste of a renewable energy plant, CO_2 production for the elevated CO_2 system was not included.

Inventory data were acquired by interviewing the farm, and unavailable data such as transport to the central wholesale market, fuel input during farming, and electricity use at distribution center were collected from previous reports [22–26] (Table 2). CO_2 emissions per 100 g of harvested spinach were calculated by multiplying inventories by CO_2 emission intensity units, as shown in Table 1.

CO_2 emission intensity units were adapted from a government report [26]. For organic fertilizer and fuel use at the farm, CO_2 emissions per 10 a were converted to those per 100 g-spinach, by dividing those by the area and the production of the model farm. Fuel was used in agricultural machines. For transport, CO_2 emissions for a one-way trip were calculated using fuel-efficiency, 10.3 km/L, and average speed, 30.2 km/h [22,23]. Transport occurred 24 times. For the distribution center, CO_2 emissions per day was converted to 24 days. Residence time in the distribution center varied, and we assumed one day on average.

Table 1. Incubator-scale cultivation of *Spinacia oleracea* of the conventional system and the elevated CO_2 treatment system.

	Conventional System	Elevated CO_2 Treatment System
Temperature (°C)	22	22
Humidity (%)	50	50
Lighting (%)	60	60
CO_2 concentration (ppm)	400	1000
Lighting time (h)	16	16

Table 2. Inventories from production of spinach at 10 a farm to distribution center. N: nitrogen; P: phosphorous; K: potassium.

Spinach Life Cycle Inputs							
	Unit	Use (per 10 a)			CO_2 emissions (kg CO_2 per unit)		
		N	P	K	N	P	K
Organic fertilizer	kg	24.0	19.8	16.9	1.15	0.86	0.15	Adapted from [24]
Fuel	L	2.5			2.7			Fuel use adapted from [22]
Transport							
	Unit	Use (per one way)			CO_2 emissions (kg CO_2 per unit)		
Truck transport	L	5.86			2.32			Fuel efficiency [20]; Average speed adapted from [21]
Distribution Center							
	Unit	Use (per day)			CO_2 emissions (kg CO_2 per unit)		
Electricity	kWh	0.9			0.5			Electricity use adapted from [23]

CO_2 emissions of the eCO_2 system was estimated by dividing the CO_2 emissions of the Conv system by the spinach weight ratio of eCO_2 system. Weight ratio was calculated by dividing the average weight of the eCO_2 system by that of the Conv system, which was measured as 2.2.

2.4. Eco-Efficiency Score

The eco-efficiency score was calculated by dividing vitamin C content by CO_2 emissions, which were measured in Sections 2.1 and 2.3, respectively. We assumed that nutritional quality would represent the value of vegetable, which would possibly enhance the economic value. Among nutrients, vitamin C was chosen due to its high content in spinach and the result of questionnaire survey [27,28].

$$\text{Eco} - \text{efficiency [score]} = \frac{\text{vitamin C content [g/100 g} - \text{spinach]}}{CO_2 \text{emission [g} - CO_2/100\ \text{g} - \text{spinach]}}$$
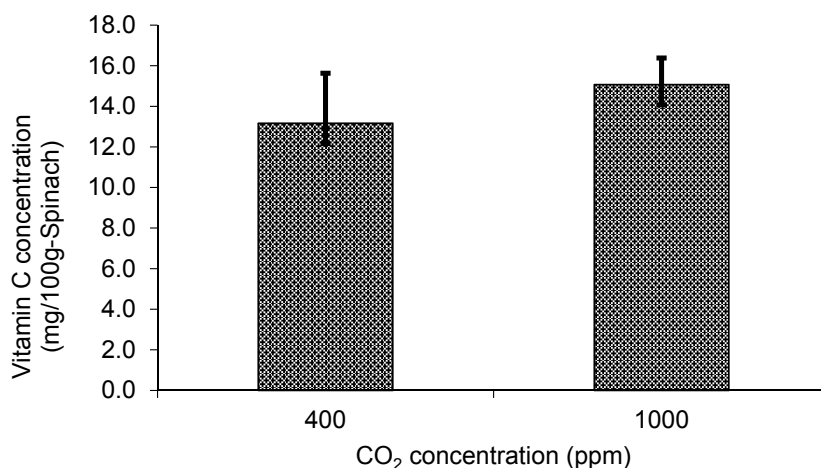
3. Results

The effectiveness of the elevated CO_2 treatment in improving the growth, nutritional quality, and environmental impact has been clarified. We compared weight, vitamin C content, and CO_2 emissions of the Conv system (400 ppm CO_2) to the eCO_2 system (1000 ppm CO_2). Additionally, the eco-efficiency score, integrating nutritional value and environmental impact, was compared between the two systems.

Figure 2 shows spinach weights at 20 days of growth under different CO_2 treatments. Weights were measured per bouquet of spinach. The averages of the Conv system and the eCO_2 system were 1.59 g and 2.70 g, respectively, showing 1.69 times higher weight in the eCO_2 system. *t*-test proved that there was a significant difference between the treatments ($p = 0.005$). Vitamin C concentration was estimated by the absorbance of a spectrophotometer, followed by calculation using a calibration curve (Figure 3). The average concentration of the Conv system was 13.2 mg/100 g spinach (standard deviation 4.27), and it was 15.1 mg/100 g in the eCO_2 system (standard deviation 2.27).
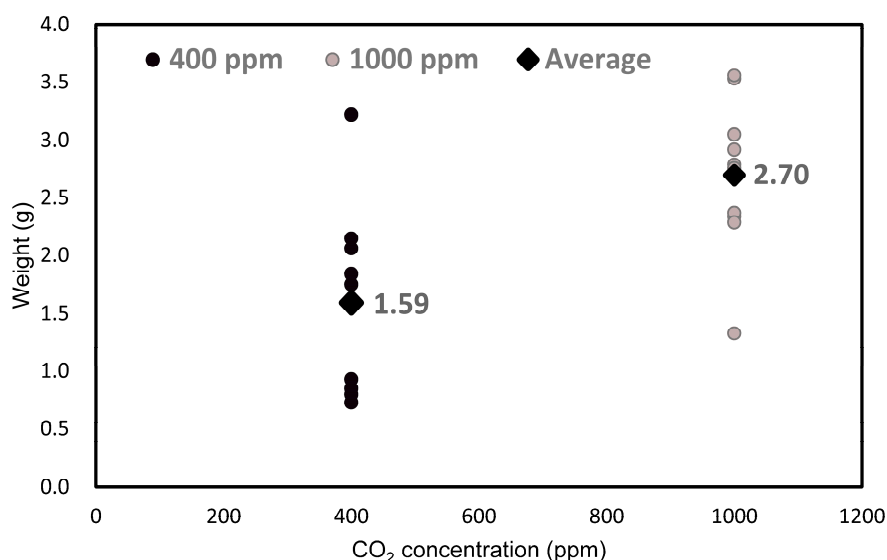

Figure 2. Weights of spinach of conventional production (400 ppm) and elevated CO_2 treatment (1000 ppm). Average of the treatments are marked with a black diamond shape.

Figure 3. Average vitamin C concentration of conventional production (400 ppm CO_2) and elevated CO_2 treatment (1000 ppm CO_2). Bars indicate the standard deviation.

CO_2 emissions were estimated by the inventory calculation result of the Conv system, the model spinach farm (Figure 4). Emissions of 49.0 g-CO_2/100 g-spinach of Conv system and 29.0 g-CO_2/100 g-spinach of eCO_2 system were obtained. The CO_2 emissions of the Conv system were 1.69-fold greater than the eCO_2 system. Closely looking into the Conv system, 2 g-CO_2/100 g-spinach for organic fertilizer, 0.3 g-CO_2/100 g-spinach for fuel, 1.5 g-CO_2/100 g-spinach for distribution center, and 45.3 g-CO_2/100 g-spinach for transport were shown to be emitted. CO_2 emission during transport of spinach from the farm to the central wholesale market accounted for about 90% of the

total. The reason for this is that the model farmer conducted organic farming, and they are doing pesticide-free cultivation. Agricultural chemicals are known as one of hot spots of CO_2 emission during cultivation [29,30].
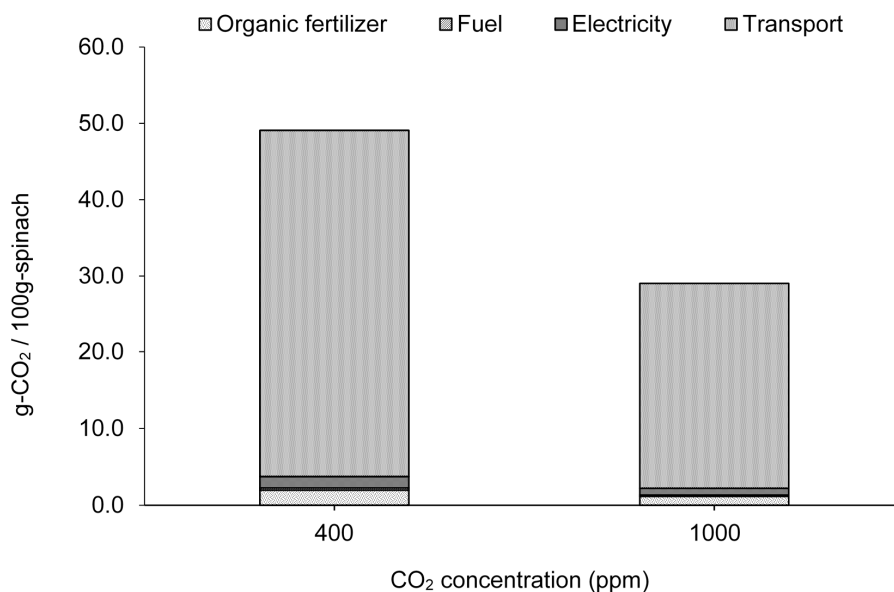
Figure 4. CO_2 emissions of conventional production system (400 ppm CO_2) and elevated CO_2 system (1000 ppm CO_2).

The eco-efficiency of the eCO_2 system was scored as 0.76, and that of the Conv system was 0.26. This demonstrates that the eCO_2 system was 2.9-fold more efficient than the Conv system.

4. Discussion

Elevated CO_2 treatment could improve the growth and environmental impact for spinach production in a protected horticulture system by using waste CO_2 from other systems as a source. It was found that the weight of elevated CO_2 treatment for 20 days was 1.69-fold greater than ambient CO_2 system. Additionally, vitamin C was relatively higher in the elevated CO_2-treated spinach, showing 14% higher than conventional production. A previous report showed similar results to this study, which compared weights and vitamin C between elevated CO_2 treatment (900 ppm) and conventional production (350 ppm). It indicated 1.65-fold heavier bouquets and 1.08-fold more vitamin C for the elevated CO_2 treatment [29]. This tendency was also reported in other vegetables. They investigated the physiological effect of elevated CO_2 treatment on vegetables, although it differed according to species [31–34]. Ripening tomatoes and bananas showed a respiratory reduction with high CO_2 treatment [35]. Eggplants, cucumbers, podded peas, spinach, and lettuce showed C_2H_4 production and respiration increase by high CO_2 [32]. Especially, plants of C_3, to which spinach (*Spinacea oleracea*) belongs, showed relatively high photosynthetic rate under elevated CO_2 [13,35]. These physiological enhancements due to elevated CO_2 could possibly induce the growth and high concentration of vitamin C as shown in this study. It is expected that productivity will be improved by developing new control methods and species in the future [36–38].

Elevated CO_2 treatment facilities often adopt natural gas to produce CO_2 or use CO_2 exhausted from other industries. In order to further abate the environmental burden of elevated CO_2 treatment, the source of CO_2 is important. Combined heat and power (CHP) systems use natural gas as a fuel, generate electricity with a gas engine, and use exhaust heat and exhaust gas, CO_2. The electricity produced at the facility is mainly used for supplementary lighting, heat is applied to heating, and CO_2 is applied to the facility to promote growth [38]. Another example is the use of CO_2 from composting

facilities. Nakano et al. showed that about 1000 ppm of CO_2 was exhausted every hour during composing tomato farm waste [39].

We would propose to use waste CO_2 from renewable energy plants. It was suggested that environmental impact could be reduced by elevated CO_2 treatment due to the growth increase, assuming that CO_2 emission to produce waste CO_2 could be ignored. It is recommended to combine a protected horticulture system with the Blue Tower process [40,41]. The Blue Tower process is a renewable energy production systems which uses biomass, and its main product is hydrogen. Nagaishi et al. estimated the CO_2 emissions of the Blue Tower process in consideration of CO_2 fertilization [42]. Results showed CO_2 emission was reduced as 36.37 g-CO_2/MJ. It was suggested that the use of CO_2 in vegetable production could reduce the total CO_2 emissions of a biomass hydrogen plant. However, the cost often hampers the use of waste CO_2 from other systems. For example, some regions in the Netherlands are using CO_2 from the oil industry, but this is not widespread due to the high cost—it costs more than natural gas [38]. In order to promote the use of waste CO_2 from renewable energy plants in protected horticulture systems, it is necessary to investigate the cost of installing and operating the plants.

Toward the sustainable development of agriculture, it is important to increase the value of vegetables while reducing environmental impact. We focused on nutrition to evaluate the value of vegetables in this study. We assumed that enhanced nutritional quality could trigger an increase in economic value. We chose to study nutritional quality as it related to CO_2 emissions. We assumed that nutrition should be an important factor, which is comparable to economic value. We assessed the eco-efficiency score, integrating nutritional value, vitamin C content, and environmental impact (CO_2 emission). Results showed that elevated CO_2 treatment was 2.9-fold more efficient than conventional production. It was deemed that elevated CO_2 treatment could be an effective production system to enhance the vegetable nutritional quality and reduce environmental burden. However, there are still challenges. The growth and vitamin C increase should be verified at the field scale, because the growth experiment in this study was performed at the laboratory scale using a plant incubator. By integrating nutritional quality and environmental burden, eco-efficiency could be a useful environmental communication tool. It is expected that it should give incentives to producers and customers to raise the environmental awareness, and further produce value-added vegetables.

5. Conclusions

It is suggested that elevated CO_2 treatment could promote the growth and nutritional quality of spinach. Further, it could contribute to CO_2 abatement in the agriculture sector due to the use of waste CO_2 and enhanced growth. The eco-efficiency score indicated that elevated CO_2 treatment of spinach could increase nutritional quality while reducing environmental burden. We believe that eco-efficiency, integrating nutrition and environmental impact, could be useful to give incentive to customers and farmers, raising environmental awareness for customers and inspiring farmers to produce value-added vegetables. This study offers important preliminary data to further strengthen the long-term sustainability of the agriculture sector, integrating environmental impact and the nutritional quality of vegetables.

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