Yield and quality of curly kale grown using organic fertilizers

Marta R Verruma-Bernardi [1]; **Daniella M Pimenta** [1]; **Gabriel RR Levrero** [1]; **Victor A Forti** [1]; **Simone DS de Medeiros** [2]; **Sandra Regina Ceccato-Antonini** [1]; **Elizabete A Covre** [1]; **Marcos D Ferreira** [3]; **Raissa Moret** [3]; **Alberto CC Bernardi** [4]; **Fernando C Sala** [1]

[1]Universidade Federal de São Carlos (UFSCar), Araras-SP, Brasil; verruma@ufscar.br; d_ni_martins@outlook.com; ga_levrero@hotmail.com; viaugu@ufscar.br; antonini@ufscar.br; betecovre@hotmail.com; fcsala@ufscar.br; [2]Universidade Federal de Santa Catarina (UFSC), Florianópolis-SC, Brasil; sisartorio@gmail.com; [3]Embrapa Instrumentação, São Carlos-SP, Brasil; marcos.david@embrapa.br; raissamoret@gmail.com; [4]Embrapa Pecuária Sudeste, São Carlos-SP, Brasil; alberto.bernardi@embrapa.br

ABSTRACT

Kale is a vegetable that has high nutrition content, and balanced fertilization is essential to ensure high yield and quality of agricultural products. Curly kale, less known than regular leaf kale, is a new possibility for consumption. However, little is known about the influence of the type of fertilization on nutritional characteristics. This study aims to evaluate the influence of using organic fertilizers on the productivity, microbiological, and physico-chemical characteristics of Darkibor hybrid curly kale. The treatments consisted of three sources of organic fertilizers, one of organomineral fertilizer, and the control (without fertilization). The highest productivity was observed for kale that was treated with fertilizer in the organomineral composition in all harvests. For microbiological analyses, there was no contamination in all treatments, following legislation. There was no significant difference between treatments for the physicochemical composition, highlighting the high levels of phenolic compounds and proteins in curly kale. There was no difference between treatments regarding the mineral content of the leaves. Organic and organomineral fertilizers made it possible to produce curly kale with adequate physicochemical composition, free from microbiological contamination and heavy metals.

Keywords: *Brassica oleracea* var. *acephala*, minerals, post-harvest, color, weight loss.

RESUMO

Rendimento e qualidade da couve crespa cultivada com fertilizantes orgânicos

A couve é uma hortaliça com bom valor nutricional e adubações balanceadas são essenciais para garantir alta produtividade e qualidade de produtos agrícolas. A couve de folha crespa, menos conhecida do que a de folha lisa, é nova possibilidade para consumo, todavia, pouco se conhece sobre a influência do tipo de adubação nas características nutricionais. O objetivo do estudo foi avaliar a influência do uso de fertilizantes orgânicos na produtividade, características microbiológicas e físico-químicas da couve de folha crespa, híbrido Darkibor. Os tratamentos foram três fontes de adubos orgânicos, uma de adubo organomineral e o controle (sem adubação). A maior produtividade foi observada para as couves submetidas ao tratamento com adubo na composição organomineral em todas as colheitas realizadas. Para as análises microbiológicas, não houve contaminação em todos os tratamentos, estando de acordo com a legislação. Não houve diferença significativa entre os tratamentos para composição físico-química, ressaltando os altos teores de compostos fenólicos e proteínas na couve de folha crespa. Quanto aos teores de minerais nas folhas não houve diferença entre os tratamentos. O uso de fertilizantes orgânicos e organomineral possibilitaram produzir couve de folha crespa com adequada composição físico-química, isentos de contaminação microbiológica e metais pesados.

Palavras-chave: *Brassica oleracea*. var. *acephala*, minerais, pós-colheita, cor, perda de massa.

Brassica plants are rich in nutrients and bioactive compounds (Baenas *et al*., 2012), highlighting proteins (Lorenz & Maynard, 1988), carbohydrates, calcium, iron, iodine, vitamin A, niacin, vitamin C (Trani *et al*., 2015) and antioxidants (Baenas *et al*., 2012). Within this genus, species of *B. oleracea* evolved in many plant varieties where popular vegetables such

as kale, brussels sprouts, cabbage, and broccoli are found essential sources of nutrients in many countries (Ordás & Cartea, 2008).

Kale is widely consumed in Brazil, especially collard, but curly kale is still little known in the consumer market. Unlike collard, curly kale, has curly leaves and is generally dark green (Olsen *et al*., 2009; Pathirana *et al*.,

2017; Bejo, 2019). Also, it is high yielding and has good tolerance to early weighing (Bejo, 2019).

Studies have shown that curly kale has high concentrations of minerals, fibers, and proteins (Thavarajah *et al*., 2016). According to Gunnars (2018), 67 g of curly kale contains seven times the daily recommendation for vitamin K, providing 3 g of protein, 6 g of

carbohydrates, of which 2 g is fiber, and 33 calories.

Vegetables are characterized by short growth cycles and high nutritional requirements. Vegetables of the genus *Brassica* have a high demand for calcium, sulfur, and boron (Filgueira, 2008), and for this reason, the supply of nutrients in a balanced way is essential to ensure high yield and quality of the vegetable. Studies have shown that organic fertilization has a positive result on the growth of these vegetables. Organic fertilizer is a product of plant, animal, or agro-industrial origin, which, when applied to the soil, improves fertility and increases crop productivity and product quality (Trani *et al.*, 2013).

Organic fertilizers are generally found in solid and liquid form. Solid fertilizers are often incorporated into the soil before planting, and these organic materials are rich in slow-releasing organic N; it is difficult to predict the rate of mineralization in planning to meet crop uptake needs (Yoder & Davis, 2020).

According to Brasil (2020), simple, mixed, compound, and organomineral organic fertilizers are classified according to their production's raw materials. Based on this normative instruction, the organic fertilizers in this research are referred to Class "A" and "B" fertilizers.

Class "A" organic fertilizer uses raw material generated from extractive, agricultural, industrial, agro-industrial and commercial activities, including those of mineral, vegetable, animal, industrial and agro-industrial sludge from wastewater treatment plants (authorized by the environmental agency), fruit and vegetable waste and food waste generated in pre- and post-consumption. These fertilizers are segregated at the generating source and collected by selective collection, free from waste or health contaminants, resulting in a product safe to use in agriculture. Class "B" fertilizer uses any amount of organic raw materials generated in urban, industrial, and agro-industrial activities in its production. It includes the organic fraction of urban solid waste from conventional collection, sludge generated in sewage treatment plants,

industrial and agro-industrial sludge generated in wastewater treatment plants containing any amount of waste or health contaminants. These fertilizers are authorized by the environmental agency, resulting in a product safely used in agriculture.

Massive production of organic waste provides Brazil with a high potential for organic fertilizers or organic mineral fertilizers (Sá *et al.*, 2017; Correa *et al.*, 2018). Organomineral fertilizers can replace mineral fertilizers; they have equivalent performance and add organic compounds to the soil, potentially improving their properties and agricultural production (Mumbach *et al.*, 2020).

The type of fertilizer used in the crop can influence the chemical composition of vegetables, affecting their biological quality (Bernardi *et al.*, 2005). Knowledge of the effect of fertilizers on the characteristics of the vegetable can help expand the options of use and reduce production costs, especially considering that there is less knowledge about curly kale than the other types. In this context, this study aims to evaluate the effect of using three organic fertilizers (two from class A and one from class B) and one organomineral (class B) on the productivity, microbiological and physicochemical characteristics of curly kale. The work's hypothesis is replacing organomineral fertilizer with organic fertilizer without impairing production, microbiological health, and the physicochemical composition of curly kale.

MATERIAL AND METHODS

Site and characteristics of the experiment

The experiment was carried out at the Center for Agricultural Sciences, at the Federal University of São Carlos (CCA/UFSCar), Araras-SP (22º21'25"S, 47º23'03"W, 646 m altitude), a region characterized by dry winters and rainy summers.

The soil in the area was a Red Latosol with the following chemical characteristics: pH $(CaCl_2)$ = 5.7;

organic matter = 33 g dm^{-3}; P = 7 mg dm^{-3}; K = 6.9 mmol dm^{-3}; Ca^{2+} = 68 mmol dm^{-3}; Mg = 15mmol dm^{-3}; Al = 0.5 mmol dm^{-3}; H+Al = 21 mmol dm^{-3}; CTC = 110.9 mmol dm^{-3}; Fe = 43 mg dm^{-3}; Mn = 23.9 mmol dm^{-3}; Zn = 4.6 mmol dm^{-3} and Cu = 3.9 mmol dm^{-3}.

The curly kale used was *Brassica oleracea* var. *acephala*, Darkibor hybrid. The seedlings were produced in trays with commercial substrate, and 30 days after germination, they were transplanted to the experimental plots in the field and irrigated using a sprinkler. The experimental plots were 12 m^2 with 50 x 50 cm spacing between plants and rows. The useful area of the plot used for the evaluations was ten central plants. The experimental design was of randomized blocks with five treatments (fertilizers and control) in four replications.

The evaluated treatments were: A) control, without fertilizer application; B) organomineral fertilization, class B [organic compound of materials for reuse and recycling of waste generated by human activities, macro and mineral micronutrients (0.7 kg/plot in planting and 0.05 kg/plot topdressed)]; C) organic fertilization 1, class A (comprising poultry litter, bone meal, industrial slaughterhouse waste, and food and other industrial waste (2.3 kg/plot in planting and 0.2 kg/plot topdressed); D) organic fertilization 2, class B [comprising sewage sludge (4.7 kg/plot in planting and 0.31 kg/plot topdressed)] and E) organic fertilization 3, class A [consisting of sugarcane filter cake (2.3 kg/plot in planting and 0.34 kg/plot topdressed)]. Information on the description of the fertilizers was the same as that described on the product label.

The quantities of fertilizers used were defined according to the soil's chemical characteristics and based on the requirements of the NPK in planting and cover fertilization (Trani *et al.*, 2015). The planting fertilizations were carried out one week before planting and immediately after the cuts, and the covering was carried out fortnightly until the 60th day, and, after that date, it was carried out monthly.

Yield assessment

Kale productivity was evaluated in

four harvests carried out at 60, 90, 120, and 150 days after planting. All the leaves of the plot's four central plants were collected, determining the mass of fresh matter by weighing.

Microbiological analyses

Microbiological analyses were performed on plants collected at 60, 90, and 120 days after planting. Initially, kale leaves were washed in running water, cut, and then immersed for 10 minutes in water with 200 mg/L sodium hypochlorite and washed again with sterile distilled water. The kale leaves were packed in sterile plastic bags, and 1 mL of 0.1% sterile peptone water was added per gram of sample. Each sample was washed by shaking the plastic bag vigorously 50 times carefully to avoid puncturing it or liquid overflow. This procedure was based on the methodology described in Paiva (2011), with modifications.

From the liquid remaining after washing the leaves, aliquots were removed to detect *Salmonella* spp. using the 1-2 Test kit (BioControl) after pre-enrichment of the samples in lactated broth at 35°C for 24 hours. The total coliforms and *Escherichia coli* analysis was performed on Petrifilm™ EC plates (3M).

Physicochemical analysis

For the physicochemical analysis, the third or fourth fully developed leaf was selected, from the apex to the plant's base (Trani *et al.*, 2015) of the four central plants.

The mass loss, turgor pressure, and instrumental color analyses were performed in the first harvest (60 days), at 0, 5, and 9 days of storage. Three packs (two leaves each) were placed in polyethylene bags (0.30 x 0.40 m) opened and stored in a refrigerated chamber at 10±1°C and relative humidity between 85-90%.

Mass loss was determined by weighing, considering the difference between the initial and final mass. The Wiltmeter® equipment was used to determine the turgor pressure loss (Calbo *et al.*, 2008). The instrumental color was evaluated using the Hunterlab colorimeter, and the following was recorded: L value (lightness), which

varies from black (L = 0) to white (L = 100); the $a*$ value, which varies from red ($+a*$) to green ($-a*$); and the $b*$ value that varies from yellow ($+b*$) to blue ($-b*$).

For the analysis of total soluble solids, pH, moisture, fibers, phenolic compounds, minerals, and heavy metals were analyzed at 60 (1st harvest), 90 (2nd harvest), and 120 (3rd harvest) days after planting, except for phenolic compounds, which were determined in the first two harvests. The total soluble solids (°Brix) were analyzed by directly reading the supernatant in a digital bench refractometer (Atago model RX-5000α-Plus). The pH was determined in the homogenized material using an Edutec bench model pot EEQ9003-110 at 20°C.

The fiber content was analyzed using the ANKOM method (2017). The moisture content was determined in an oven at 100-105°C until constant mass; the ash content was obtained by the burning method at 600-650°C (AOAC, 2012). The total phenolic compounds (gallic acid equivalent per 100 g) were determined according to the Folin-Ciocalteu spectrophotometric method with modifications (Singleton & Rossi, 1965).

To determine the minerals (potassium, calcium, magnesium, sulfur, boron, copper, iron, manganese, and zinc) and contaminants (arsenic, cadmium, chromium, and lead), 0.5 g of each sample were digested in nitric-perchloric acid (7 mL). After digestion, the samples were diluted to 25 mL using deionized water and stored in plastic tubes at room temperature. Mineral composition and contaminants were analyzed by inductively coupled plasma optical emission spectrometry (ICP-AES, Varian®). The nitrogen analyses were carried out by Kjedhal method in hot sulfuric acid extract (Nogueira *et al.*, 2005).

Statistical analysis

For the total soluble solid variables, pH, moisture, fibers, phenolic and mineral compounds, ANOVA was applied separately for each harvest considering a completely randomized design with five treatments and four

replications. For the longitudinal variable (yield), a structure was considered to distribute treatments in subdivided plots, in which the plots have the treatments and in the subplots, the time (days after planting).

For the instrumental color variables (L, $a*$, $b*$), mass loss, and turgor pressure, ANOVA was performed considering a completely randomized design in a subdivided plot scheme, with five treatments (A, B, C, D, and E) and in the subplots three times (0, 5 and 9 days), with six repetitions.

The verifications of the technique assumptions were carried out and, when necessary, the Scott-Knott means comparison test was applied for the treatments. All analyses were performed using the R Core Team software (R Core Team, 2020), considering a significance level of 5%.

RESULTS AND DISCUSSION

Yield

Regarding the production of fresh mass, the interaction was significant between time and treatment, indicating that these factors are dependent. Evaluating the developments (Table 1; Figure 1), there was a significant increase in the curly kale yield with the organomineral source (B) supply in the four crops analyzed, with the highest averages.

In the first harvest (60 days), all treatments differed ($p \leq 0.05$) from each other, in which the control (A) had the second-lowest average fresh mass production, which was higher only than the organic fertilizer E (comprising sugarcane filter cake). At 90 days, organic fertilizer D (sewage sludge) showed the lowest average fresh mass production, and organic fertilizers C and E were similar to the control (A). For the harvest (120 days), the organomineral fertilizer was superior to all the others. At 150 days after planting, the production of the control treatment (A) showed the lowest average, and the three organic fertilizers (C, D, and E) did not differ from each other, and treatment B (organomineral) showed the highest average fresh mass production

(Table 1; Figure 1). The increase in the fresh mass yield with the organomineral source is probably due to the greater immediate availability of nutrients in the soil from the mineral fraction of the fertilizer, while the organic fraction may have influenced the improvement of the physical structure of the soil in the long term. A similar effect was discussed by Mumbach *et al.* (2020).

In all the analyzed crops, the organomineral fertilizer (B) yield was between 12 and 34% higher than the control treatment (A). The differences between the organomineral source (B) and the organic sources (C, D, and E) were more significant in the first harvest (60 days), with differences between

8 and 57%. At 150 days, despite the tendency to decrease productivity in all treatments, the organomineral source yield remained between 9 and 13% higher than the organic sources (C, D, and E). This positive response to the supply of organic fertilizers in kale was also observed by Balcãu *et al.* (2012) and Haile & Ayalew (2018).

Microbiological analysis

Microbiological analysis results showed that the kale from all treatments in the three harvests met the standard established by Brazil (2019) and were absent for *Escherichia coli*. There was also no contamination by total coliforms or thermotolerant coliforms or by *Salmonella* spp. from any fertilization

treatment; that is, the results are under the legislation that establishes the absence of *Salmonella* spp. in 25 g of sample.

Analyzing 65 samples of certified organic vegetables, Maffei *et al.* (2013) did not find *Salmonella* spp. in the samples. However, they found thermotolerant coliforms in 41.5% of organic vegetables. Machado *et al.* (2006) also did not find *Salmonella* spp. in vegetables grown under different organic fertilizers.

Physicochemical analysis

There was no significant interaction between the evaluation and treatment days for the variables of mass loss, turgor pressure, and instrumental color (L, *a**, and *b **), indicating that these factors are independent. Thus, the factors were assessed separately (Table 2; Figure 2), and there was no influence on the type of fertilizer on the parameters evaluated.

There was a significant effect during storage, with paraboloid behavior for the variables mass loss, turgor pressure, and color *a** and *b**, increasing mass loss (Figure 2a) and *b** (Figure 2e). For the instrumental color L parameter (Figure 2c), there was a linear behavior, with a 1.09 increase in lightness each day, regardless of the treatment.

Between the fourth and fifth days, a maximum point for turgor (350 Kpa) was observed for kale regardless of the treatment applied. Readings close to zero refer to withered leaves, while the most extensive readings are from leaves not subject to dehydration (Ferreira & Calbo, 2008). Evaluating turgor in kale, Ferreira & Calbo (2010) found 372 Kpa, higher than the values observed in this study, which ranged from 329.41 Kpa to 339.37 Kpa for kale grown using organic fertilizers. The turgor of vegetables is a parameter that indicates the plant quality, in the case of leafy plants, that is, the consumer does not well accept leafy plants with signs of wilting.

Mass loss was obtained for each unit of days, as shown in the second-degree equation in Figure 2a. Studying kale stored in plastic packaging at 10°C, Simões (2004) reported a 15% mass loss on the 2nd day of storage.

Table 1. Averages of fresh mass production (kg ha^{-1}) for each treatment compared to the evaluated time. Araras, UFSCar, 2019.

DAP	A (control)	B (organomineral)	C (organic)	D (organic)	E (organic)
60	27419.9 d	36698.7 a	28997.3 c	33766.7 b	23297.6 e
90	23330.6 b	26199.2 a	22807.9 b	21210.0 c	23985.0 b
120	18369.6 b	22922.0 a	18764.7 b	18515.6 b	18381.3 b
150	18599.0 c	22428.5 a	19873.3 b	19702.8 b	20482.6 b

Averages followed horizontally by different letters differ (p≤0.05) by the Scott-Knott test. DAP = days after planting. CV $_{plot}$ = 3.3198%; CV$_{subplot}$ = 3.9071%. A: control, without fertilizer application; B: organomineral fertilization, class B: organic compound of materials for reuse and recycling of waste generated by human activities, macro and mineral micronutrients; C: organic fertilization, class A: comprising poultry litter, bone meal, industrial slaughterhouse waste, and food and other industrial waste; D: organic fertilization, class B: comprising sewage sludge; E: organic fertilization, class A: comprising sugar cane filter cake.

Table 2. Treatment averages for mass loss (ML), turgor pressure (TP), and instrumental color (L, *a** and *b**) in curly kale grown with organomineral and organic fertilizers. Araras, UFSCar, 2019.

Parameter	A (control)	B (organomineral)	C (organic)	D (organic)	E (organic)
ML (%)	12.54 a$^{\#}$	8.90 a	9.62 a	10.06 a	15.01 a
TP (Kpa)	331.00 a	325.60 a	329.4 a	331.71 a	339.38 a
L	38.60 a	38.90 a	36.0 a	36.70 a	38.10 a
a*	-4.80 a	-4.20 a	-4.8 a	-5.30 a	-4.10 a
b*	10.90 a	11.60 a	9.2 a	9.80 a	11.50 a

Averages followed by different letters in the lines differ (p≤0.05) by the Scott-Knott test. L = Lightness (0 = black and 100 = white); *a** = red/green coordinate (+a indicates red and –a indicates green); *b** = yellow/blue coordinate (+b indicates yellow and –b indicates blue). A: control, without fertilizer application; B: organomineral fertilization, class B: organic compound of materials for reuse and recycling of waste generated by human activities, macro and mineral micronutrients; C: organic fertilization, class A: comprising poultry litter, bone meal, industrial slaughterhouse waste, and food and other industrial waste; D: organic fertilization, class B: comprising sewage sludge; E: organic fertilization, class A: comprising sugar cane filter cake.

Post-harvest losses of vegetables may be related to physiological factors, mechanical damage, or parasites. The accumulated post-harvest losses on vegetables are estimated to range between 20 and 50% (Finger & França, 2011). Due to the high metabolism of kale, its leaves are highly perishable, with signs of yellowing and loss of turgor, causing a consequent short post-harvest period (Sanches *et al.*, 2016).

According to Novo *et al.* (2011), aspects of appearance such as size, shape, brightness, and leaf color are the main quality attributes observed by the consumers when buying leafy vegetables. The leaf color attribute is of fundamental importance, as the consumer decides to buy the product based mainly on the appearance, associating it with a freshness indicator without considering the texture, nutritional value, and flavor. Vegetables with greener and brighter leaves are recommended for consumption by professionals in nutrition and gastronomy (Henz & Mattos, 2008).

The analysis of total soluble solids, pH, moisture, fibers, ash, and phenolic compounds of curly kale is shown in Table 3. The average levels of total soluble solids varied in the three harvests but showed no significant
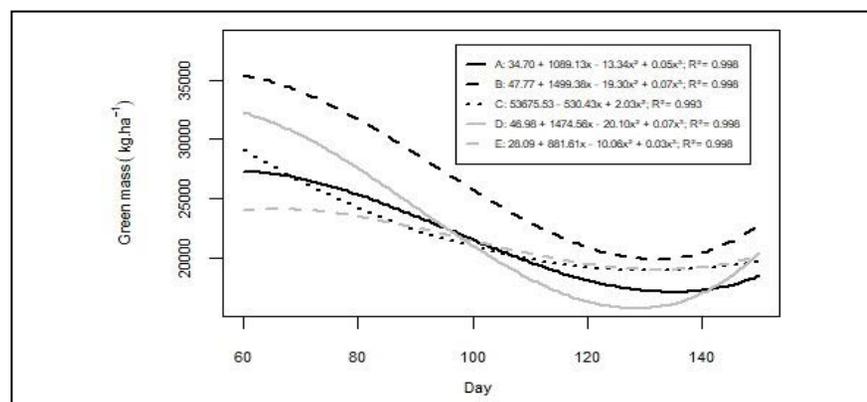

Figure 1. Estimated curves, by orthogonal polynomials, for each treatment over the days after planting to produce green mass of curly kale. A: control, without fertilizer application; B: organomineral fertilization, class B: organic compound of materials for reuse and recycling of waste generated by human activities, macro and mineral micronutrients; C: organic fertilization, class A: comprising poultry litter, bone meal, industrial slaughterhouse waste, and food and other industrial waste; D: organic fertilization, class B: comprising sewage sludge; E: organic fertilization, class A: comprising sugar cane filter cake. Araras, UFSCar, 2019.

Table 3. Average of total soluble solids (TSS), pH, humidity, fibers, ash, and phenolic compounds (PC) in curly kale leaves grown with different fertilizer compositions. Araras, UFSCar, 2019.

Parameters	DAP*	Treatments					CV(%)
		A (control)	B (organomineral)	C (organic)	D (organic)	E (organic)	
TSS (°Brix)	60	7.11a*	6.81a	8.20a	7.41a	7.88a	10.69
	90	9.12a	9.12a	8.50a	8.03b	7.71b	5.01
	120	6.96a	5.85a	5.07a	6.01a	6.06a	15.26
pH	60	5.65a	5.66a	5.62a	5.62a	5.63a	0.93
	90	6.19a	5.93b	5.99b	6.01b	6.04b	0.53
	120	6.40a	6.47a	5.55b	5.80b	6.36a	5.66
Moisture (%)	60	85.56a	85.54a	85.34a	85.76a	85.66a	0.49
	90	85.71a	85.35a	85.82a	86.27a	85.51a	0.87
	120	85.51a	85.43a	86.10a	85.82a	85.33a	0.76
Fiber (%)	60	4.00a	3.99a	3.34a	4.33a	3.66a	11.54
	90	3.53a	4.43a	3.94a	3.92a	4.34a	9.42
	120	3.70a	3.68a	3.93a	3.88a	3.46a	12.92
Ash (%)	60	1.40a	1.32a	1.35a	1.35a	1.33a	5.66
	90	1.98a	2.32a	2.31a	2.39a	2.31a	7.13
	120	2.56c	2.23b	2.07b	2.56a	2.22b	6.23
PC (mg EAG per 100g)	60	657.33a	586.97a	643.06a	712.24a	609.63a	10.94
	90	851.64a	658.26a	802.77a	810.48a	888.65a	19.02

Averages followed by distinct letters in the lines differ (p≤0.05) by the Scott-Knott test. DAP = days after planting. CV (%) = Coefficient of variation. A: control, without fertilizer application; B: organomineral fertilization, class B: organic compound of materials for reuse and recycling of waste generated by human activities, macro and mineral micronutrients; C: organic fertilization, class A: comprising poultry litter, bone meal, industrial slaughterhouse waste, and food and other industrial waste; D: organic fertilization, class B: comprising sewage sludge; E: organic fertilization, class A: comprising sugar cane filter cake.

difference between treatments. Sanches *et al.* (2016) found a value of 7.8°Brix in collard leaves. The authors reported that production factors, such as genetic material used, soil type, climate, and cultural treatments, might affect this parameter.

The average pH of the treatments was in the range of 5.62-6.47 and showed significant differences between some treatments, however, according to Menezes *et al.* (2005), the ideal pH range for vegetables is between 5.0 and 7.0. Sanches *et al.* (2016) obtained a pH of 5.20 in collard.

Concerning kale moisture, the levels varied between 85.33 to 86.27%, with no statistical difference between treatments, which were equivalent to the values already described by Lorenz & Maynard (1988) of 85% for curly kale.

Kale presented between 3.34 to 4.34% fiber, with no difference between treatments. Curly kale leaves had a
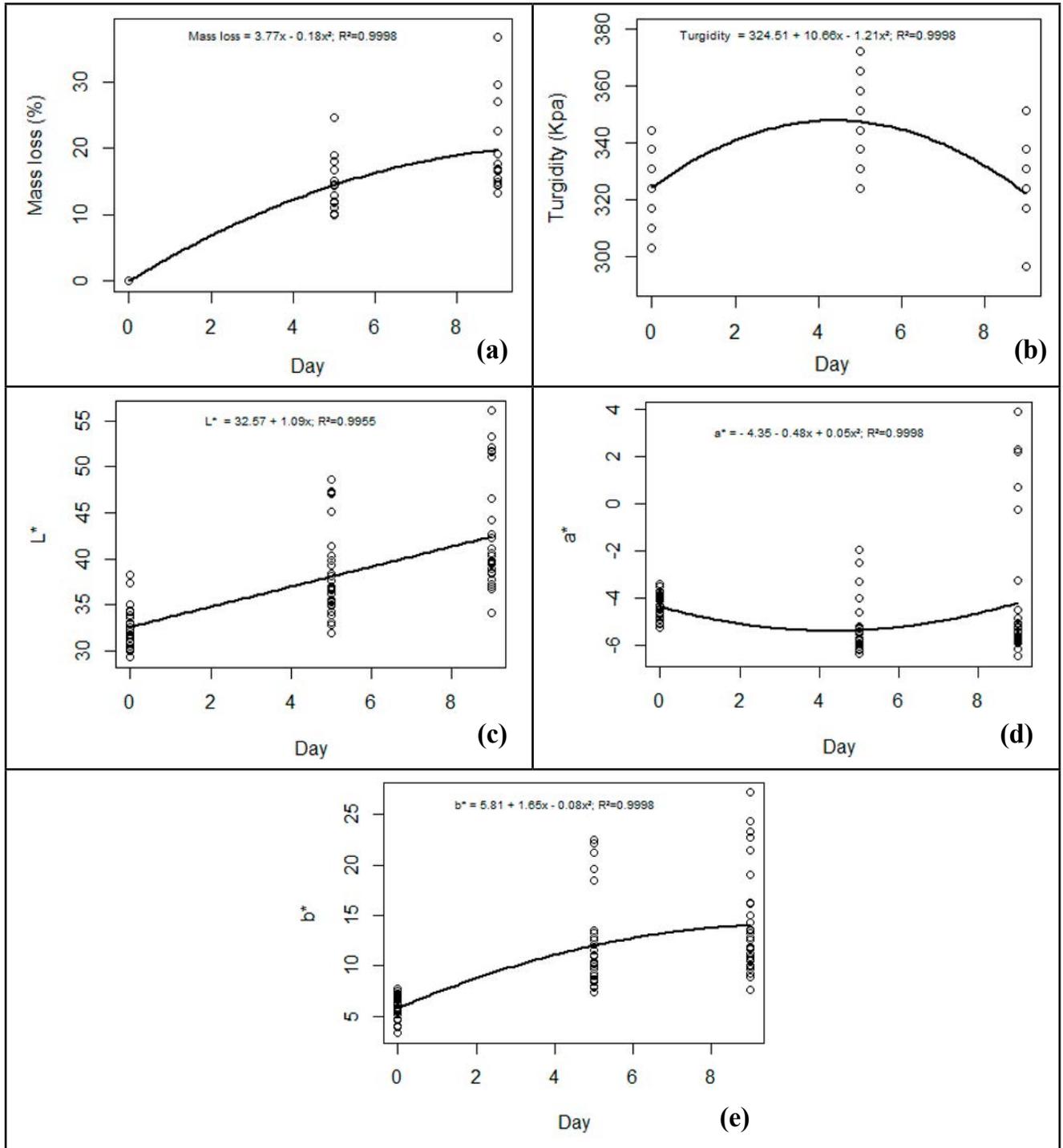

Figure 2. Behavior described by orthogonal polynomials of the parameters: (a) mass loss (ML%); (b) turgor pressure; (c) instrumental color L; (d) instrumental color *a**; (e) instrumental color *b** over the days of curly kale leaves were stored. Araras, UFSCar, 2019.

higher fiber content than collard, which had 1.3 g (Luengo *et al.*, 2011) and 2.0 g/100 g (Lorenz & Maynard, 1988).

The phenolic compounds' content varied between 586.97 and 888.65 mg EAG per 100 g, with no statistical difference between treatments. There was an increase in values for all treatments from the first to the second harvest. The values obtained for curly kale were higher than those found by Rigueira *et al.* (2016), who obtained phenolic compounds between 173 to 244 mg per 100 g in leaves of another variety of collard. These differences occur since the comparison is made with different varieties. In addition to the fact that collard has a higher moisture content, a dilution of the values of solids and phenolic compounds, can probably occur.

The ash content in kale varied between harvests; higher values

were found in the 2ⁿᵈ and 3ʳᵈ harvest. Compared with the collard with 1.3% (Taco, 2011), the curly kale's ash contents are higher.

Analyzing nutrients in kale leaves, the averages of treatments in the three harvest seasons did not show statistically significant differences (p≥0.05) for any analyzed minerals (Table 4). The levels of N, B, Cu, and Fe within the range of values are considered adequate by Trani *et al.* (2015) for kale, in all treatments used.

The N levels in the three harvests are within the range considered adequate by Trani *et al.* (2015). The values of protein content ranged from 25.2 to 31.6 g kg⁻¹, calculated from the total N content (Table 4). For comparison, Thavarajah *et al.* (2016) obtained values between 16 and 59 g kg⁻¹ by studying kale cultivars; and Thavarajah *et al.* (2019) found values between 13 and 60 g kg⁻¹ in kale

grown in an organic system.

In all the averages, the P levels were higher than the limits considered adequate by Trani *et al.* (2015) in the first harvest (60 days). The levels in all treatments of the second and third harvests were in the range considered adequate and were equivalent to the values observed by Luengo *et al.* (2018).

The K contents of the treatments with the different fertilizers are within the appropriate range. However, the control treatment showed values above those considered adequate for K in the first harvest (60 days). In the other harvests (90 and 120 days), the values were within the appropriate ranges.

In the first harvest, the Ca levels were below the appropriate range (Trani *et al.*, 2015) in the control treatments, fertilized with organomineral (B) and organic (C and D) fertilizers. Only treatment with organic fertilizer E was in the range

Table 4. Average macronutrient analysis in the kale leaves grown with organomineral and organic fertilizers compared to Trani *et al.* (2015). Araras, UFSCar, 2019.

Paramters	DAP	Treatments					CV(%)	Trani *et al.* (2015)
		A (control)	B (organomineral)	C (organic)	D (organic)	E (organic)		
N (g kg⁻¹)	60	44.50a	50.50a	49.25a	48.00a	46.75a	7.20	
	90	43.80a	42.95a	44.08a	42.28a	42.03a	4.06	30 - 55
	120	40.94a	44.16a	45.31a	43.24a	40.25a	6.03	
P (g kg⁻¹)	60	7.28a	7.72a	7.81a	7.71a	7.21a	6.01	
	90	4.31a	5.12a	5.07a	4.76a	4.70a	5.94	3 - 7
	120	3.88a	4.32a	4.03a	4.52a	4.23a	11.35	
K (g kg⁻¹)	60	43.25a	40.03a	39.61a	29.53a	31.67a	12.35	
	90	29.70a	38.98a	35.93a	34.85a	34.25a	28.36	20 - 40
	120	22.86a	37.49a	29.51a	35.75a	25.83a	24.51	
Ca (g kg⁻¹)	60	12.92a	12.30a	10.77a	13.95a	15.47a	29.26	
	90	24.47a	25.68a	24.28a	22.38a	22.75a	8.07	15 - 25
	120	15.95a	15.70a	22.31a	17.89a	21.81a	18.96	
Mg (g kg⁻¹)	60	2.36a	2.15a	2.10a	2.10a	2.46a	6.70	
	90	3.45a	3.14a	3.55a	3.12a	3.26a	6.05	3 - 7
	120	2.11a	2.20a	2.92a	2.63a	2.65a	14.32	
S (g kg⁻¹)	60	10.37a	10.61a	10.89a	8.94a	10.68a	6.85	
	90	9.03a	5.54a	10.24a	10.79a	10.88a	32.77	-
	120	6.25a	7.54a	7.42a	7.67a	5.40a	13.61	

Averages followed by distinct letters in the lines differ (p≤0.05) by the Scott-Knott test. A: control, without fertilizer application; B: organomineral fertilization, class B: organic compound of materials for reuse and recycling of waste generated by human activities, macro and mineral micronutrients; C: organic fertilization, class A: comprising poultry litter, bone meal, industrial slaughterhouse waste, and food and other industrial waste; D: organic fertilization, class B: comprising sewage sludge; E: organic fertilization, class A: comprising sugar cane filter cake.

Table 5. Average analysis of micronutrients and contaminants in kale leaves grown with organomineral and organic fertilizers compared to Trani *et al.* (2015). Araras, UFSCar, 2019.

Parameters	DAP	Treatments					CV(%)	Trani *et al.* (2015)
		A (control)	B (organomineral)	C (organic)	D (organic)	E (organic)		
B (mg kg⁻¹)	60	46.5a	53.5a	43.0a	45.5a	51.5a	14.85	
	90	24.0a	20.0a	18.5a	17.0a	30.5a	44.12	30-100
	120	27.0a	14.5a	17.5a	14.5a	14.1a	28.14	
Cu (mg kg⁻¹)	60	12.5a	13.5a	13.5a	13.5a	13.5a	8.57	
	90	11.0a	10.5a	12.5a	12.5a	6.5a	29.83	5-20
	120	7.1a	5.5a	5.5a	6.0a	6.5a	13.71	
Fe (mg kg⁻¹)	60	223.0a	196.0a	250.5a	222.0a	213.0a	20.8	
	90	183.0a	290.5a	232.5a	196.5a	219.0a	16.80	60-300
	120	257.0a	194.0a	152.0a	301.0a	211.5a	38.45	
Mn (mg kg⁻¹)	60	27.0a	23.5a	24.5a	24.5a	27.0a	10.53	
	90	24.5b	27.1a	23.5b	23.0b	22.5b	3.48	40-250
	120	22.5a	26.0a	22.5a	22.5a	22.0a	14.68	
Zn (mg kg⁻¹)	60	26.5a	32.0a	36.0a	44.5a	31.5a	20.96	
	90	26.0a	27.0a	26.0a	25.0a	23.0a	12.20	30-150
	120	29.0a	26.0a	19.0a	30.5a	21.0a	20.66	
As (mg kg⁻¹)	*	<LD	<LD	<LD	<LD	<LD	-	-
Cd (mg kg⁻¹)	*	<LD	<LD	<LD	<LD	<LD	-	-
Cr (mg kg⁻¹)	*	<LD	<LD	<LD	<LD	<LD	-	-
Pb (mg/kg⁻¹)	*	<LD	<LD	<LD	<LD	<LD	-	-

Averages followed by distinct letters in the lines differ (p≤0.05) by the Scott-Knott test. * 60, 90, and 120 days. A: control, without fertilizer application; B: organomineral fertilization, class B: organic compound of materials for reuse and recycling of waste generated by human activities, macro and mineral micronutrients; C: organic fertilization, class A: comprising poultry litter, bone meal, industrial slaughterhouse waste, and food and other industrial waste; D: organic fertilization, class B: composing sewage sludge; E: organic fertilization, class A: composing sugar cane filter cake.

considered adequate. However, from the second and third harvests (90 and 120 days), the secondary macronutrient's values were in the appropriate range. These adequate values are in agreement with Luengo *et al.* (2011), who observed calcium levels in kale of 25.0 g kg⁻¹ and Brussels sprouts of 18.2 g kg⁻¹.

The average leaf levels of Mg were below the limits of the appropriate range for all treatments studied in the harvest at 60 days, but were in the appropriate range in the harvest at 90 days and decreased again to values below adequate in the last harvest (120 days). Luengo *et al.* (2018) also observed leaf levels of Mg below the critical level in 61% of the properties analyzed.

For sulfur, there are no reference values in Trani *et al.* (2015). However, it is an essential nutrient for brassicas, as it participates in the composition of the cysteine and methionine amino acids and presents high extraction (Cantarella & Montezano, 2010). The nitrogen:sulfur ratio varied from 4.2:1 to 5.3:1 for the treatments evaluated in the first harvest (60 days) and, 3.9:1 to 7.8:1 in the second harvest (90 days), and 5.6:1 to 7.5:1 in the third harvest (120 days). These ratios are below the values generally found in plant tissues, between 12-15:1 (Cantarella & Montezano, 2010). With a higher amount of sulfur in relation to nitrogen, these relationships indicate that the sulfur present in the soil was sufficient to meet the nutritional requirements of kale.

The decreasing order of the macronutrient contents in kale leaves was: N>K>Ca>S>P>Mg, in which nitrogen is the most extracted nutrient, followed by potassium and calcium. This result differs from previous studies conducted with brassicas, in which

greater potassium extractions were observed in kale leaves (Aquino *et al.*, 2009; Correa *et al.*, 2013). However, these results are in agreement with Takeishi *et al.* (2009) for leaf, stem, and cauliflower inflorescence. The sulfur contents were higher than those of phosphorus, and these were higher than those of magnesium. In studies carried out by Aquino *et al.* (2009), Takeishi *et al.* (2009), and Correa *et al.* (2013), sulfur contents were higher than phosphorus and magnesium.

In the first harvest, the B values in the leaves were within the appropriate range (Table 5), and from the second and third harvests (90 and 120 days), the values of this micronutrient were below the appropriate range. Luengo *et al.* (2018) also observed that most of the kale samples from properties in the Federal District were below the appropriate range proposed by Trani

et al. (2015). Probably N reduced B concentration in the tissues as there is an antagonism in the absorption of B by the increase in the applied N (Mengel & Kirkby, 2001; Rodrigues et al., 2009).

The Cu and Fe levels in the three evaluation periods and all treatments and control (Table 5) were within the appropriate ranges proposed by Trani et al. (2015). Mn average leaf contents were below the limits of the appropriate range for all treatments studied in all evaluation periods. These levels below the adequate one were also observed by Luengo et al. (2018) in samples of different properties. The reduction in Mn absorption can be explained by the presence of N, preferably in the form of nitrate in well-drained soils such as the experimental area. The absorption of this anion leads to an increase in rhizosphere's pH, which can result in decreased absorption of metallic cations such as Mn and Zn (Mengel & Kirkby, 2001).

Zn content was below the adequate level for the control treatment in the first harvest (60 days) and in the appropriate range on the other treatments. However, after harvest at 90 and 120 days, all Zn levels were below the range considered adequate. These results indicate a negative interaction between the absorption of P and Zn, as the excess of the absorption of the macronutrient (Table 5) inhibited the absorption of the micronutrient as already demonstrated by Mengel & Kirkby (2001).

Regarding micronutrients, the decreasing order of leaf contents was Fe>B>Zn>Mn>Cu. These results are in agreement with the contents presented by Luengo et al. (2018).

There is a growing interest in green leaves that contain higher concentrations of Zn, and that can provide a substantial source of this element (Broadley et al., 2010). Zn plays an essential role in blood clotting and protecting DNA from modification, decreasing the risk of cancer (Messias et al., 2015). Brasil (2005) recommends that the daily amount consumed by a healthy adult individual is between 9 to 59 mg of Fe and 7 mg of Zn. Therefore, 100 g of fresh kale would correspond to 35% of the minimum amount of Fe and 7% of the recommended value of Zn.

The contaminating heavy metals (arsenic, cadmium, chromium, and lead) were not detected in curly kale leaves grown using different fertilizers. This indicates that the sources used had no contaminants that could harm the final quality of the vegetable.

The organomineral fertilization promoted more outstanding production of curly kale leaves, regardless of the harvest season. Organic and organomineral fertilizers made it possible to produce curly kale with adequate physicochemical composition, free from microbiological contamination and heavy metals.

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