Article

The Impact of Organic Fertilizer Produced with Vegetable Residues in Lettuce (*Lactuca sativa* L.) Cultivation and Antioxidant Activity

Tamara Righetti Tupini Cavalheiro 1, Raquel de Oliveira Alcoforado 1,2, Vinicius Soares de Abreu Silva 3, Pedro Paulo Saldanha Coimbra 1, Nathânia de Sá Mendes 1, Elisa D’avila Costa Cavalcanti 1, Diogo de Azevedo Jurelevisc 1 and Édira Castello Branco de Andrade Gonçalves 1,4,*

1 Laboratory of Bioactivities, Graduate Program in Food and Nutrition (PPGAN), Federal University of the State of Rio de Janeiro (UNIRIO), Rio de Janeiro 22290-240, Brazil; tamara_righetti@hotmail.com (T.R.T.C.); ragirl150@gmail.com (R.d.O.A.); coimbra.nut@gmail.com (P.P.S.C.); nathimendes@hotmail.com (N.d.S.M.); elisadcco@gmail.com (E.D.C.C.)
2 Environmental Sciences, Federal University of the State of Rio de Janeiro (UNIRIO), Rio de Janeiro 22290-000, Brazil
3 Laboratory of Biotechnology and Microbial Ecology. Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro 21941-901, Brazil; viniciussas1998@gmail.com (V.S.d.A.S.); diogo@micro.ufrj.br (D.d.A.J.)
4 School of Nutrition, Department of Food Science, Federal University of the State of Rio de Janeiro (UNIRIO), Rio de Janeiro 22290-000, Brazil
* Correspondence: ediracba.analisdealimentos@unirio.br

Abstract: Large amounts of organic waste are produced worldwide. This work aims to evaluate the influence of organic fertilizers, onion peel flour (OPF) and fruit and vegetable flour (FVF) on lettuce cultivation. Lettuce seeds were planted in pure soil (P), P with 10% OPF, and P with 3% FVF. Soil and lettuce leaves were analyzed. The addition of OPF and FVF improved soil quality but only in the soil with FVF was there significant lettuce growth and increase of antioxidant activity: Folin (8.89 mg EAC/g FM), FRAP (1.31 mg Fe2+/g FM), ABTS (4.09 mg TEAC/g−1 FM) and ORAC (0.52 mg TEac/g−1 FM). The present results show an improvement in lettuce nutritional quality and the potential of FVF as an organic fertilizer.

Keywords: plant waste; lettuce; total phenolic content; ABTS; FRAP; ORAC

1. Introduction

Globally, the amount of organic waste generated is approximately 1.3 billion tons per year, with 670 million tons generated by developed countries and 630 million tons generated by developing countries. Brazil produces more than 240,000 tons of waste per day, with 76% of this amount deposited in landfills throughout the country, of which 60% is organic waste [1]. These residues represent an economically viable and abundant raw material for use in different processes, such as the production of biochemicals, biomaterials, and energy [2].

Onion skin is one of the primary residues generated during processing and this residue is used as a supplement in bread production, quercetin purification, and pigment extraction [3].

Onion peel has a large amount of carbohydrates (82.15%), a smaller amount of protein (3.06%), ashes (5.93%), and fiber (7.78%), and a high content of biopolymers, approximately 93% [4]. Considering total dietary fibers (TDF), the onion peel has the most significant amount of these components, and insoluble dietary fibers (IDF) are its main fraction (1:13; SDF:IDF) with 41.1% α-cellulose, 16.2% hemicellulose, and 38.9% lignin [5]. Moreover, onion peel has a notable antioxidant capacity (76% inhibition of DPPH) [6].
After the production of an isotonic drink with integral use of three fruits and eight vegetables, a large amount of solid residue was generated [7]. These residues underwent a drying procedure to obtain the fruit and vegetable flour (FVF). A high content of biopolymers (approximately 84%), mainly composed of dietary fibers (48%), where 9.6% are soluble dietary fibers (SDF) and 39% insoluble dietary fibers (IDF) in a ratio of 1:4 SDF:IDF, has been described for this flour [8] soluble and insoluble fiber fraction, showing amounts of soluble (4.4 g/100 g) and insoluble (14.9 g/100 g), cellulose (19.1 g/100 g), hemicellulose (6.5 g/100 g), and resistant starch (0.7 g/100 g) with a particle size range within 212–300 μm [9]. Significant amounts of digestible carbohydrates (26%), proteins (9.5%), lipids (5%), and ashes (4.9%) were also found [8], adding to a remarkable antioxidant capacity (61% DPPH inhibition) [10], and 88 phenolic compounds, which include phenolic acids (28%), flavonoids (32%), and other polyphenols (28%) [11].

The application of these residues in soil has the advantages of increasing organic matter content, water retention capacity, buffering the soil acidity, and stimulating microbial activity, characterizing its application as an organic fertilizer [12]. This practice has been widely used in many countries with benefits for soil physiochemical and biological properties, increasing fertility and higher nutrient availability such as nitrogen (N), phosphorus (P), potassium. (K), calcium (Ca), and iron (Fe) [13].

Lettuce (Lactuca sativa L.) is the most popular leafy vegetable in terms of consumption and so is of great importance in the human diet [14]. Due to a large number of varieties, it can be successfully cultivated throughout the year [15], with a high production potential, especially using organic fertilizers [16]. From a nutritional point of view, it is rich in ascorbic acid, vitamins A and K, folic acid, carotenoids, and other bioactive substances, mainly phenolic compounds (0.2 mg EAG/g in fresh mass (FM)) with a significant antioxidant capacity of approximately 1.79 mg TEAC/g FM, 1.15 mg TEAC/g FM, and 0.0250 mg TE/g, evaluated by FRAP, ABTS, and ORAC methods, respectively [17–19]. The ideal temperature for lettuce cultivation is between 15 and 18.5 °C, however it can withstand temperatures as low as 5 °C, and warm temperatures can lead to rapid plant growth and early flowering. The ideal soil pH for lettuce cultivation ranges from 6.0 to 6.8 [20].

The intensive use of soil, specifically those focused on vegetable production, causes a reduction in organic material and nutrients and has been identified as one of the most important threats to its quality [13]. Thus, the purpose of this study was to evaluate the influence of organic fertilizers produced from vegetable residues on Crespa lettuce cultivation.

2. Materials and Methods

The experiment was carried out in the greenhouse of the Health Sciences Center (CCS) of the Federal University of Rio de Janeiro (UFRJ) from 22 March to 19 June 2019. Irrigation was carried out continuously (Monday to Friday) with an average of 7 uninterrupted irrigations for 15 minutes a day. Each irrigation provided approximately 18 to 20 mL of water per sowing.

2.1. Preparation of Organic Fertilizer from Onion Peel Flour (OPF)

Onion hulls were obtained after minimal processing of onions from 4 food and nutrition units located in Botafogo, and 3 Hortifruti units located in Botafogo and Copacabana, Rio de Janeiro/Brazil. The material was transported in polyethylene bags to the Laboratory of Bioactives where they were dried in a ventilated oven (Marconi, Model MA035, Brazil) at 65 °C for 5 h; grounded in a blender (Philco, 900 W); then returned to the ventilated oven at 90 °C for 1 h [21]. The produced OPF was stored in sealed aluminum bags at room temperature.

2.2. Soil Preconditioning

Black soil fertilized with worm humus from the Minhocário Pé da Serra brand was conditioned with 10% OPF and 3% FVF from the mixture by geometric dilution and kept in the greenhouse without irrigation for 3 days.
2.3. Experimental Design

Sowings (126 mL/cell capacity) were filled with pure soil (P), P + OPF 10%, and P + FVF 3%. A total of 18 seedlings was prepared for each treatment, and each one received 4 lettuce seeds of the Crespa Grand Rapids TBR variety, from the Isla manufacturer. It was established that transplantation of all sowings would be done when one of the cultivation conditions reached a lettuce leaf height between 8 and 10 cm [22] and the harvest reached about 22 cm [23]. The pots (6 for each treatment) used for transplantation were preconditioned as described above and then kept in the greenhouse under the same irrigation conditions for 30 days before this stage. Soil analyses were made, considering time 0, as described below. The same analyses were done in the soil of 6 seedlings, which were randomly collected in the transplant, and the lettuce leaves were evaluated for antioxidant capacity. Soil samples from all pots were also analyzed at the final harvest, as well as lettuce leaves.

All treatments were randomly distributed and under the same conditions.

2.4. Soil Analyses

2.4.1. Sample Collection

The samples were randomly chosen and each sowing was entirely collected, packed, and homogenized in polyethylene bags. Samplings were made in the morning, before or after the first irrigation. Samples collected before irrigation were homogenized and stored in sealed dark-colored polyethylene bags for pH and soil basal respiration (SBR) analysis. Samples collected after irrigation were homogenized and stored in falcon tubes for moisture (U) and water retention capacity (WRC) analyses. The physicochemical analyses were done in triplicate.

2.4.2. Moisture

The samples were placed in falcon tubes properly sealed with parafilm and stored under refrigeration for 24 h. The moisture content was performed with the Gehaka 2500 Infrared (IR) analyzer and Ohaus digital IR analyzer.

2.4.3. Water Retention Capacity

25 g of the samples were transferred to a plastic funnel and sealed with glass wool. The samples were saturated with distilled water and capped with plastic wrap to prevent evaporative water loss. After 4 h, the water-saturated samples were placed in a parafilm-sealed falcon tube and refrigerated for 48 h. The moisture was then measured using the Ohaus digital IR analyzer and Gehaka 2500 IR analyzer [24].

2.4.4. Soil Basal Respiration

20 g of each sample was placed in 50 mL polypropylene flasks and incubated in dark-colored polyethylene bags for 7 days. To the same incubation bag was added a similar vial containing 5 mL of 1M NaOH. Following incubation, NaOH was titrated with 0.5 M HCl using the phenolphthalein indicator [25]. The SBR was calculated as Equation (1), where “vol. A” is the volume of HCl spent on sample titration (mL), “vol. B” is the volume of HCl spent on blank titration (mL), “M” is the molarity of HCl solution, “dm” is the sample dry mass (g), and “T” is the incubation time (hours).

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\text{SBR (mg C-CO2 / kg dry soil / h)} = (\text{vol. B} - \text{vol. A}) \times \text{M dm / T}
\]

2.4.5. pH

The samples were placed in sealed polyethylene bags and stored under refrigeration for 72 h. The pH was measured in a Biovera pH meter in a soil:distilled water (1:2) mixture [26].
2.4.6. Analysis of Lettuce Leaves

Lettuce height was measured in centimeters (cm) from ground level to the top of the leaf [27].

2.4.7. Sample Preparation

The lettuces were collected, washed with distilled water, placed in sealed dark-colored polyethylene bags, and transported to the laboratory in dry ice (<4 °C). The leaves where dried in a ventilated oven at 65 °C for 5 h, then crushed in a blender and returned to the ventilated oven for another hour at 90 °C [21]. For the antioxidant capacity analysis, the dried leaves were extracted with a mixture of ethanol: water (50/50, v/v) in a proportion of 3.0% (w/v) and kept in shaker incubator (NovaTecnica) at 30 °C for 10 h. After this time, the mixture was centrifuged (Thermo Fisher Scientific, MegaFuge 16R, Waltham, MA, USA) at 2000 xg for 15 minutes and filtered on filter paper. The supernatant was recovered and stored at −20 °C until analysis [10]. An aqueous solution (10%, v/v) of the sample extract was used for the antioxidant capacity analysis described below.

2.4.8. Antioxidant Activity

The total phenolic content was determined using the Folin–Ciocalteau technique [28], using the Victor Nivo Microplate Reader (Perkin Elmer, Rodgau, Germany), and the results were expressed as mg of gallic acid equivalent per gram of sample (mg EAG/g⁻¹). The antioxidant activity of the ABTS radical was performed using the Victor Nivo Microplate Reader (Perkin Elmer, Germany) [29]. Results were expressed as mg Trolox per gram sample (mg Trolox/g⁻¹). Iron reducing oxidant activity (FRAP) was performed using the Victor Nivo Microplate Reader (Perkin Elmer, German) [30]. Results were expressed as mg reduced iron per gram of sample (mg Fe²⁺/g⁻¹). The antioxidant activity by ORAC was performed using the Victor Nivo Microplate Reader (Perkin Elmer, German) [31]. Results were expressed as mg Trolox equivalent per gram of sample (mg TE. g⁻¹).

2.4.9. Statistical Treatment

All analyses were performed in triplicate. Results were expressed as mean ± standard deviation. Parametric statistics were applied. For comparison of arithmetic means, analysis of variance (ANOVA) and the Tukey test were performed using GraphPad Prism version 5, with a significance level of 5% probability (p < 0.05). Pearson’s correlation analysis was applied, using statistic version 7 with a significance level of 5% probability (p < 0.05).

3. Results

3.1. Soil Quality

The variation of climatic conditions is shown in Figure 1, describing the 86 days of the experiment, with a maximum temperature of 33.8 °C corresponding to the sowing day and a minimum of 18.3 °C during the transplantation period.

![Figure 1. Variation of maximum and minimum temperatures (°C), relative moisture (%), and rainfall (PPT) during the 86 days of the experiment (Source: INMET).](image)

Temperature influences the availability of water and nutrients to plants, as it can affect the rate of water evaporation and inactivation of enzymes produced by soil
microbiota; increasing temperature can reduce the binding force between mineral particles and water and can result in reduced void space [32].

The use of FVF promoted differentiated growth of lettuce leaves (Figure 2). At transplantation, performed after 56 days of cultivation, only the soil with FVF added presented leaf height between 8 and 10 cm [22], a parameter established for this stage. Although the lettuce did not grow as expected in all treatment, there were no errors during the experiment. All treatments were randomly distributed and under the same conditions. However, to avoid misunderstandings, we minimized the difference in lettuce growth under the different treatment conditions.

Similar results related to the low growth of lettuce planted in soil with the addition of onion residues [13] was related to the high C/N ratio of onion residues.

The presence of biopolymers affects the rearrangement of soil structure. Another related factor is the composition of the organic matter added to the soil, which can influence this process positively or not, considering that amino acids and sugars are more easily decomposed while others, such as lignin and lipids, are slowly degraded [5,33]. OPF has a high lignin content (38.9%) and SDF:IDF ratio (1:13) when compared to FVF – 19.3% and 1:4, respectively [4,8]. These data suggest that maybe OPF biopolymers are slowly decomposed, negatively influencing soil nutrition and quality concerning lettuce leaf growth.

![Figure 2](image-url)

**Figure 2.** Height of lettuce during transplant and harvest, grown in soils: pure (P), P added with 10% onion peel flour (OPF), and P added with 3% fruit and vegetable flour (FVF). Different letters indicate statistical difference in the same cultivation period (p < 0.05; Tukey’s test).

Transplanting is characterized as an essential stage for plant growth, because through it the seeds can germinate faster with a good root system, and it is usually done between 25 to 40 days, with adequate root development and harvest [34]. Lettuce should be harvested 30 days after transplantation, completing the cultivation cycle [23]. In this study, the cultivation cycle comprised 86 days, considering the harvest done after 30 days of transplanting.

In the present study, the cultivation time until transplantation was delayed, which could be explained by the high temperature during this period of the experiment [20]. It is believed that high temperatures during most of the cultivation may have negatively influenced P and P + OPF 10% conditions, promoting little lettuce growth. Plant growth may be affected by some soil factors such as the distribution of the root and its ability to capture water and nutrients, which is influenced by the soil’s structure [35].

It is well known that for better cultivation, micronutrients and soil pH should be balanced [36]. However, considering the ash content of FVF (4.9%) and OPF (5.9%), we chose not to adjust the different soils proposed in this study. Thus, it was observed that even with unbalanced soil and high temperature during the cultivation period, FVF promoted...
leaf growth and maintained the cultivation cycle of this vegetable in the standards established by the literature, approximately 90 days [13].

The composition of biopolymers in organic fertilizers also impacted moisture content (Figure 3) and water retention capacity (Figure 4). The difference in soil water content influences water availability for plants and microorganisms but also has a significant effect on nutrient diffusion rate [37]. Only the soil with OPF added showed a significant increase in moisture content, keeping it at approximately 55% throughout the experiment. The addition of organic matter to the soil corresponds to the formation of pores, which can be classified as micro or macropores; their more significant interaction may cause increased aggregate stability through polysaccharides that act as binding agents [38].

![Figure 3. Soil moisture content with different treatments: pure (P), with 10% onion peel flour (OPF) added, and with 3% fruit and vegetable flour (FVF) added during lettuce cultivation during sowing periods (day 0), transplantation (day 56), and harvest (day 86). Lowercase letters indicate the difference of each treatment over time and uppercase letters indicate the difference between treatments at each time (p < 0.05; Tukey test).](image)

![Figure 4. Water holding capacity (WHC) of soil with different treatments: pure (P), with 10% onion peel flour (OPF) added, and with 3% fruit and vegetable flour (FVF) added during lettuce cultivation during sowing periods (day 0), transplantation (day 56), and harvest (day 86). Lowercase letters indicate the difference of each treatment over time and uppercase letters indicate the difference between treatments at each time (p < 0.05; Tukey test).](image)

The soil with OPF added, despite holding a high moisture content—about 60% at transplant and 50% at harvest—promoted weak lettuce growth, as previously seen. The high water content is related to restricted oxygenation, impacting plant growth [39]. In addition, low oxygenation is associated with increasing anaerobic microorganisms in the soil. An example of this group of microorganisms is denitrifying bacteria, which consist of microorganisms that promote the reduction of nitrate to nitrogen gas, causing the decline of nitrogen [40]. Other detrimental effects on anaerobic conditions happen, such as...
anoxic root stress, and the production of organic acids and hydrogen sulfide, among other toxic substances for the vegetable [41].

In the soil with the addition of FVF, WRC showed no change in growing periods, with a similar response to P soil. WRC is related to soil structure [42]. Possibly, this result is related to the plant demand for organic matter and higher nutrient absorption, negatively influencing the rearrangement of the aggregates and reducing the adsorption capacity of water.

The maintenance of soil WRC during cropping periods with the addition of FVF is possibly related to the greater need for water to maintain proper plant growth since it was the only treatment that had effective lettuce growth [35]. Through the evapotranspiration effect of the plant, the water contained in the soil is absorbed through the root, which causes alteration of the soil structure stability by shear strength changes, causing WRC alteration [43].

The soil with OPF addition had the highest WRC. The high insoluble fiber content of this matrix, represented mainly by cellulose and lignin, is related to a slower decomposition rate, providing higher water adsorption capacity by the hydrophilic groups of these biopolymers, such as carbonyls and hydroxyls [36].

Soil WRC between 10–20% resulted in lower plant growth, and root biomass compared to soil with WRC ranging between 30–50% [44]. The addition of the studied organic fertilizers reflects the opposite behavior, where soil with lower WRC (P + FVF) promoted higher plant growth.

Regarding soil metabolic activity, the most positively impacted condition was the addition of OPF (Figure 5). Despite the low growth of lettuce with this organic fertilizer, the high content of this material’s biopolymers and the proportion (10%) added to the soil seems to have stimulated increased soil metabolic activity [45].

![Figure 5. Soil basal respiration (SBR) with different treatments: pure (P), P with 10% onion peel flour (OPF) added, and P with 3% fruit and vegetable flour (FVF) added during the cultivation of lettuce during sowing (day 0), transplant (day 56), and harvest (day 86). Lowercase letters indicate the difference of each treatment over time and uppercase letters indicate the difference between treatments at each time (p < 0.05; Tukey test).](image)

FVF’s high content of phenolic compounds [8] and high antioxidant activity [21] better justify the metabolic activity of this soil at sowing, considering that soluble phenolic compounds influence microbial activity [46]. Interestingly, during the experiment, the variation of SBR with this organic fertilizer was irrelevant.

Considering the positive influence of the increased humidity during the transplantation period for P and OPF soils, the significant increase of SBR is understood. Proper soil water content is of utmost importance for nutrient diffusion, as already mentioned. With no nutrients available in P soil, the SBR rate decreases at the planting level, characterizing a lack of diversity in substances released by the root to nourish the microbiota [36].

The pH increase (See Figure 6) occurred in all soils during the experiment, but only the soil treated with FVF showed similar behavior to the study mentioned above. Organic waste applied to soils reduces acidity, justifying that the organic waste matrix contains
compounds that can chelate protons, causing the release of hydroxyl-OH clusters that buffer the medium [47].

Figure 6. Soil pH with different treatments: pure (P), with 10% onion peel flour (FCC) added and with 3% fruit and vegetable flour (FFH) added during lettuce cultivation during sowing periods (day 0), transplant (day 56), and harvest (day 86). Lowercase letters indicate the difference of each treatment over time and uppercase letters indicate the difference between treatments at each time (p < 0.05; Tukey test).

Pearson’s correlation analysis was applied using soil moisture content (SM), water holding capacity (WHC), and soil basal respiration (SBR) as parameters. Pearson’s correlation was significant in all conditions and directly proportional: SBR / WHC (r = 0.7040) SBR / SM (r = 0.677).

3.2. Lettuce Characteristics

As previously discussed, in soil with 10% OPF added and P soil, lettuce did not significantly grow, so only lettuce cultivated in soil with 3% FVF was analyzed. There was a significant increase in the antioxidant activity of lettuce cultivated in soil with FVF when compared to the control (P) (Figure 7). A variation of the total phenolic content for lettuce produced in the conventional form (0.4 mg EAG/g FM) and the organic system (0.2 mg EAG/g FM) [18]. This study presents a phenolic content higher than those mentioned, thus favoring the nutritional aspect of this vegetable.

Figure 7. Antioxidant activity of lettuce leaves cultivated in soil with 3% FVF at transplant (day 56) and harvest (day 86) and reference samples [18,19,48] expressed as total phenolic (mg gallic acid/g fresh weight (FW)), FRAP (mg Fe²⁺/g⁻¹ FW), ABTS (mg Trolox/g⁻¹ FW), and ORAC (Trolox mg/g⁻¹ FW) were evaluated separately in each analysis. Different letters indicate the statistical difference evaluated for the same method between cultivation periods and reference data (p < 0.05; Tukey test).
Lettuce analyzed by FRAP resulted in 1.78 mg Fe²⁺/g FM and lettuce grown in soil with FVF 1.31 mg Fe²⁺/g, showed no relevant difference in the results found in both studies with this method.

When lettuce was analyzed, antioxidant activity by the ABTS method shows 1.15 mg TEAC/g FM [19]. While in this study, ABTS results were four times higher, corroborating that the addition of FVF in the soil improved the nutritional value of vegetables.

Antioxidant activity results are possibly associated with the high content of bioactive compounds (88 identified phenolic compounds) observed in FVF [11] and the structure of the aggregates that favored the nutrient flow between soil and plant [18].

Higher antioxidant activity in transplantation may be associated with plant stress at this critical stage of development, considering that plants react to environmental conditions through physiological changes as defense mechanisms to different stress conditions [49].

The data obtained by the Folin and ABTS methods indicated a significant difference in lettuce transplantation and harvesting periods. However, for the FRAP method, this was not observed. Primary organic acids of lettuce are citric acid and malic acid, which in turn did not show high antioxidant activity when analyzed by FRAP and showed higher antioxidant activity when analyzed by ABTS [50]. More than 90% of the antioxidant activity analyzed by the ORAC method is attributed to hydrophilic compounds, thus compounds with lipophilic characteristics, such as carotenoids and vitamin E, are not adequately identified, which could explain the inferior antioxidant values found by this method in the present samples [51].

There is a strong correlation (r = 0.99) of antioxidant activity in lettuce transplantation and harvesting periods, and, as expected, a tendency to reduce it.

Our data suggest that FVF can be used as a biostimulant since it promoted a nutritional enhancement of lettuce and increased plant growth. Additional studies that promote changes in aggregate structure, such as the use of enzymatic treatment to facilitate decomposition and even nutrient flow, may be an alternative to boost the performance of this matrix [52].

4. Conclusions

The vegetable residue FVF was successfully utilized as organic fertilizer for Crespa lettuce cultivation. Lettuces cultivated in soil with FVF added showed improved antioxidant capacity and higher total phenolic content than reported in the literature, indicating a better nutritional profile. The addition of FVF did not significantly alter the soil moisture, water holding capacity, or pH, but did increase the soil basal respiration, indicating an augmentation of microbial activity. The other evaluated vegetable residue, OPF, showed increased soil moisture, water holding capacity, and soil basal respiration, but lettuce did not develop well in the presence of this material, possibly because of an excessive concentration of phenolic compounds found in OPF. All these results indicate that FVF, currently discarded as a residue, can be an interesting organic fertilizer and plant biostimulant to produce nutritionally enhanced lettuce.

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