Antioxidants in processed fruit, essential oil, and seed oils of feijoa

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Abstract

The degradation of nutraceutical properties during processing of the fruits of feijoa (Acca sellowiana), and the characterization of seed oils, and volatile compounds were evaluated. In feijoa fruit dehydrated by a standard convective air process, the total phenols and total flavonoids declined 42%, and the antioxidant capacity determined by ABTS, DPPH, and FRAP declined 26% with respect to lyophilized fruit. In feijoa jam, the reduction of total phenols and flavonoids was 52%, and the reduction in antioxidant capacity was 72%. Vitamin C in the jam was also reduced by the processing. Feijoa seeds had 69.4% unsaturated fatty acids, mainly linoleic (46.2%) and linolenic (3.7%) acids. Behenic acid was also detected in the seeds but in small amounts (0.91%). The feijoa skin had 31 volatile compounds in two orchards with different climate, one in a tropical highland and the other in a temperate zone. The extraction yield was on average 0.45%. The major compounds in the essential oil of the feijoa skin were 3-hexen-1-yl benzoate, elixene, spathulenol, D-germacrene and alpha-cadinol. In general, the concentration of volatile oils was higher in the temperate zone.

Keywords: Acca sellowiana; linoleic acid; nutraceutical foods; phenols in jam; volatile oil

Introduction

Feijoa fruit has several human health properties (Weston, 2010), such as antidepressant (Mahmoudi et al., 2015), and a reducer of toxicity by Mercury in blood (Tortora et al., 2019). The feijoa is a new fruit tree crop cultivated in tropical highlands of Mexico (González-García et al., 2018). Usually, the feijoa is grown in areas cooler than tropical highlands, for example, in New Zealand (Sun-Waterhouse et al., 2013), or Russia (Belous et al., 2014).

The processing of feijoa into new functional foods is popular in New Zealand, Australia, Colombia (Zotarelli et al., 2012), and Brazil (Amaral et al., 2019). It has been initiated in Mexico the processing of feijoa then studies on changes in total phenols and antioxidant capacity in processed fruit is required to improve benefits for humans, and to conserve bioactive compounds.
There is little information regarding changes in antioxidant content after feijoa is transformed into jam, and dehydrated fruit (Buratto et al., 2019). Beyhan et al. (2010), showed that air-dried feijoa fruit had adequate antioxidant capacity, but losses in bioactive compounds by this process were not specifically mentioned.

Essential oils are volatile substances of an organic nature, responsible for the aroma of plants, and they have medicinal properties. The volatiles of the feijoa skin have antimicrobial and pharmacological properties (Smeriglio et al., 2019). Some of the volatile compounds in feijoa are methyl and ethyl benzoates, which represent approximately 90% of the volatile fraction and are responsible for the aroma of the fruit (Binder and Flath, 1989). The production of essential oils in the skin of the feijoa is affected by environmental conditions (Basile et al., 2010; Smeriglio et al., 2019), and by the cultivars (Peng et al., 2019). It is unknown the yield capacity of the feijoa skin to produce essential oils when it is grown in tropical highland conditions.

The composition of the oils in the seeds of feijoa may vary according to the geographical origin of production (Weston, 2010).

In the present study, the antioxidant activity and total phenols and total flavonoids in dehydrated fruit, and jam were evaluated. The oils in the seeds, and essential oil in the skin of feijoa fruit were also determined.

**Materials and Methods**

**Plant material and growth conditions**

Feijoa (*Acca sellowiana* (O. Berg) Burret) fruit was collected at physiological maturity in Huatusco, Veracruz, Mexico, in July 2015 to study the effects of dehydration. The fruit was harvested from eight-year-old trees propagated by seed. The trees were under rain-fed conditions. The trees were established in a 4 × 4 m planting arrangement in a tropical highland at 1950 m elevation (19°11′12.48″ N, 97°11′13.75″ W), with average annual rainfall and temperature of 1825 mm and 17 °C, respectively. On these trees Feijoa fruit was also collected for the essential oil determination. In addition, fruit were collected in an orchard located in a temperate zone in Chapingo, Texcoco, State of Mexico. This orchard had feijoa seedlings at 4 × 4 m. This orchard was located at 2250 m (19°29′05″ N, 98°53′11″ W). The average annual temperature and rainfall was 16 °C and rainfall 618 mm, respectively. The trees had irrigation in Spring and Summer. For the essential oil determination, two fruits per each of five trees in each of the two different orchards were used in July 2017.

**Dehydrated fruit**

Fruit was lyophilized (Labconco° 4.5) for 9 h at a pressure of 332 Mbar*10^{-3} and at -41 °C or dehydrated in an air convection oven (Sedona Combo-SD-P9150-F) at 60 C for 8 h and afterwards at 40 °C for 24 h. In each case, 50 fruits were used.

Thus, in a batch of 30 fruit replicated five times in each of the two treatments, 0.500 g of a mix of dehydrated pulp or skin fruit were weighed, subsequently grounded and placed in falcon tubes, mixed with 10 mL of a methanol/water solution (4: 1, v/v). This mixture was homogenized in a vortex at 3000 rpm, 3 min, and subsequently the pH was adjusted to 3 ± 0.05 with 5% HCl solutions. The mixture was stirred for 15 minutes (Ultrasonic Cleaner 8890, Cole-Parmer) and stirred (30 min) at 27 °C. Finally, it was centrifuged (2500 rpm, 15 min) (SOLBAT J-600, Mexico). The supernatant was made up to 10 mL with the methanol/water solution (4: 1, v/v). Total phenols, total flavonoids and antioxidant capacity were quantified with the obtained solutions.

**Jam elaboration**

Jam was elaborated from a quantity of 500 g of fruit (skin + pulp). Half (250 g) was chopped into pieces, and the rest was ground in a blender (Oster 4125). The blended material was mixed with the fruit pieces and emptied into a pewter pot. Sugar was added, together with 1 g of high-methoxyl pectin (Dupont°); this mixture was heated to a temperature of 60 °C. The fruits:sugar percentage was 60:40. Five replications were made. For
the extraction, 1 g of feijoa jam was weighed and all the steps for extraction were as mentioned before. Total phenols, total flavonoids and antioxidant capacity were quantified.

The vitamin C in the jam was determined by the volumetric method (Association of Official Analytical Chemists [AOAC], 1990). An extractive solution was prepared by mixing 7.5 g of metaphosphoric acid, 200 mL of distilled water, and 20 mL of acetic acid. Jam samples were analyzed in triplicate for each of the five jam replications.

**Assays of bioactive compounds**

The content of total phenolic compounds in the extracts of the feijoa tissues was determined using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Gallic acid was used for constructing the standard curve at 765 nm. The polyphenol concentration in the samples was derived from a standard curve of gallic acid. The total flavonoids (mg EC g$^{-1}$ FW) were determined according to Kubola and Siriamornpun (2011). The catechin calibration curve to determine total flavonoid content (mg EC g$^{-1}$ DW) was in a range from 5.8 to 290 μg mL$^{-1}$. These evaluations were adapted to microplates.

For determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) (μmol ET g$^{-1}$ DW) radical scavenging activity, a mixed solution of 2.5 mg of DPPH radical with 100 mL of methanol was used (Cheng et al., 2006). For the reaction, 3.9 mL of DPPH radical was placed in a test tube, and 100 μL of extract was added. The mixture was shaken and kept for 30 minutes in the dark at room temperature. Absorbance was measured at 517 nm. Results were calculated in Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent antioxidant capacity. Radical-scavenging activity (RSA) was calculated as a percentage of DPPH decoloration, using the equation RSA% = (1-Am/Ar) 100, where Am is the absorbance of the solution when the sample extract is added at a particular level, and Ar is the absorbance of the DPPH as control.

The inhibitory activity of the ABTS ([2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (μmol equiv Trolox g$^{-1}$ DW) cation radical on the extracts was measured according to Re et al. (1999). The ABTS was prepared by mixing 7 mM ABTS with 2.45 mM K$_2$S$_2$O$_8$. The mixture was kept for 16 hours at room temperature in the dark until it reached a stable state. Each day of the analysis, the ABTS solution was diluted with distilled water to an absorbance of 0.700±0.02 at a wavelength of 734 nm. A mixture was made of 980 μL of ABTS solution and 20 μL of the fruit pulp, and absorbance was determined at 734 nm after 8 minutes using a Du 7500 UV spectrophotometer (Beckman). The blank was 80% methanol. A calibration curve was plotted using Trolox as the standard (within a range of 7.5 to 65 mM) in a methanol/water solution (80:20 v/v), under the conditions described above.

The FRAP (Ferric Reducing Antioxidant Power) assay (μmol equiv Trolox g$^{-1}$ DW) was carried out according to Benzie and Strain (1996) and adapted to a 96-well microplate. Each well contained 20 μL of the tissue extracts, 180 μL of the FRAP solution, and 60 μL of distilled water. The plate was subsequently shaken for 30 min, and absorbance was measured at 600 nm in a microplate reader (Synergy 2 Microplate reader, Biotek International, software Gen5). A blank of 260 μL of the FRAP solution was used. The Trolox calibration curve was in a range of 3.8 to 46.0 μM. The reducing power of the feijoa extracts was estimated from the equation obtained by the curve.

**Seed oils**

A total of 30 g of seeds was extracted from randomly harvested fruit. Oil from the seeds was obtained using a Soxhlet distillation instrument (Meyer and Terry, 2008). The oil from the seeds was derivatized to fatty acid methyl esters (FAMEs), which were analysed using an Agilent 6890 gas chromatograph with a flame ionization detector (FID) using an AT-Silar column (López-Yerena et al., 2018).

**Essential oils in the fruit skin**

It was isolated from the fruit skin by hydro distillation using the Clevenger device (Shaw et al., 1983). There were 10 replication fruit per each of the two studied zone. The essential oil was dissolved in HPLC grade
hexane at a 1/1000 ratio and 1 µL was injected into GC (7890 A, Agilent Technologies, Palo Alto, CA, USA) coupled with MS (Agilent 240) with ion trap detector. A VF-5 MS column (5% inert phenylmethylpolysiloxane 30 m x 250 µm x 0.25 µm). Oven temperature was set to 60 °C for 4 min, then to 150 °C at 5 °C/min, to 170 °C at 2 °C/min, and finally to 270 °C at 15 °C/min for 5 min. Helium was used as carrier gas at a flow rate of 1.0 mL/min. An n-alkane mixture (C8-C20, Sigma ®) was injected under the same conditions as the sample. The retention times of the sample components and n-alkane blend were used to calculate the Linear Retention Index (LRI) (Van Den Dool and Kratz, 1963). Compounds were identified by comparing the calculated LRI with the LRI reported in the webbook of National Institute of Standards and Technology (NIST).

Data analysis
For the dehydration fruit treatments, and jam data, the standard errors were determined. The fatty acids in the seeds, and the essential oils in the fruit skin of two different zones are expressed in %. The Infostat software was used to analyse all the data (Di Rienzo et al., 2016).

Results
Dehydrated fruit
In dehydrated or lyophilized fruit, the skin had almost two- or three-times higher contents of total phenols, total flavonoids, and antioxidant capacity obtained by DPPH, ABTS, and FRAP than the pulp (Table 1). Sun-Waterhouse et al. (2013) noted that the skin of feijoa had more bioactive compounds than the pulp. The total flavonoid content in the pulp of the lyophilized feijoa (Table 1) was three times higher than the values reported by Pasquariello et al. (2015).

Table 1. Content of total phenols (TP), total flavonoids (TF), and antioxidant capacity by ABTS, DPPH, and FRAP in skin and pulp of feijoa after lyophilization and dehydration by air convection.

|       | TP (mg GAE g⁻¹ DW) | TF (mg EC g⁻¹ DW) | ABTS (µmol ET g⁻¹ DW) | DPPH (µmol ET g⁻¹ DW) | FRAP (µmol ET g⁻¹ DW) |
|-------|--------------------|-------------------|------------------------|------------------------|------------------------|
| **Lyophilized** | | | | | |
| Skin  | 30.88 ± 2.08        | 21.41 ± 0.48      | 247.96 ± 2.85          | 151.23 ± 0.14          | 190.30 ± 1.72          |
| Pulp  | 13.72 ± 0.73        | 8.43 ± 0.42       | 120.94 ± 1.59          | 48.36 ± 0.18           | 61.38 ± 1.34           |
| **Dehydrated** | | | | | |
| Skin  | 19.38 ± 1.11        | 11.72 ± 0.36      | 172.09 ± 1.57          | 110.27 ± 0.39          | 114.07 ± 2.30          |
| Pulp  | 8.14 ± 0.40         | 4.18 ± 0.49       | 90.73 ± 2.20           | 55.61 ± 0.41           | 47.56 ± 1.72           |

When the values of antioxidant capacity in the feijoa pulp were transformed into fresh weight, they averaged for ABTS 19.92 ± 0.27 µmol ET g⁻¹ FW; this antioxidant capacity was similar to macadamia nut (Macadamia integrifolia), caimito (Pouteria caimito), arazá (Eugenia estipitata), and umani (Poraqueiba serícea) (Contreras-Calderón et al., 2011). In the skin, the antioxidant activity values obtained by ABTS (54.07 µmol ET g⁻¹ FW) and FRAP (41.63 µmol ET g⁻¹ FW) were higher than those determined in the skin of naranjilla (Solanum quitensis) and pejibaye (Bactris gasipaes) (Contreras-Calderón et al., 2011).

The feijoa dehydrated by convection with respect to lyophilized had a reduction in total phenols and total flavonoids in the skin of about 37% and 45%, respectively, and in the pulp, the reduction averaged 45% for phenols and flavonoids (Table 1). Antioxidant capacity also declined an average of 32% in the skin and 20% in the pulp (Table 1). In contrast, in other studies (Beyhan et al., 2010), the antioxidants in the feijoa pulp dehydrated by convention were less affected. Recent studies shown that the spray drying method is adequate to
conserve bioactive compounds in feijoa used as powder (Buratto et al., 2019). A convective multi-flash drying process for producing dehydrated crispy feijoa fruits could also be used (Zotarelli et al., 2012).

The conservation of bioactive compounds in the feijoa is required because the raw material must contain high content of bioactive compounds for elaboration of innovative feijoa products, such as those that stimulate immunity activity with isoflavonoids (Lapčík et al., 2005), sun screen formulations (Ebrahimzadeh et al., 2014), and medications to prevent gastritis and ulcers (Monforte et al., 2014).

**Jam**

The total phenols and flavonoids in the extract of jam (skin + pulp) (Table 2) compared to the lyophilized pulp declined 52% on average (Tables 1 and 2). The reduction in antioxidant capacity averaging ABTS, DPPH, and FRAP was 72% (Tables 1, 2). The concentration of vitamin C also declined (0.11 mg g⁻¹ DW). For example, in lyophilized feijoa skin, the values of vitamin C were about 0.59 mg g⁻¹ DW (González-García et al., 2018). The processing significantly affects the concentration of vitamin C in guava jam (Ordóñez-Santos and Vazques-Riascos, 2012). Methods of preparing feijoa jam without a significant reduction in bioactive compounds and vitamin C are required. For example, high pressure processing (Srinivas et al., 2018).

**Table 2.** Total phenols (TP), total flavonoids (TF), and antioxidant capacity by ABTS, DPPH, FRAP, and content of vitamin C in feijoa jam. Mean ± standard errors are shown

|          | TP (mg GAE g⁻¹ FW) | TF (mg EC g⁻¹ FW) | ABTS (µmol ET g⁻¹ FW) | DPPH (µmol ET g⁻¹ FW) | FRAP (µmol ET g⁻¹ FW) | Vitamin C (mg g⁻¹ DW) |
|----------|-------------------|------------------|-----------------------|----------------------|----------------------|---------------------|
|          | 1.86 ± 0.14       | 0.47 ± 0.05      | 1.43 ± 0.14           | 7.71 ± 0.56          | 11.75 ± 1.07         | 0.11 ± 0.00         |

**Oil content of the seeds**

The oil content was 0.23%, and this value was two times lower than that reported by Andrade et al. (2012) in Brazil with feijoa trees grown under temperate conditions. Linoleic acid achieved the highest value at 46% (Table 3), and Andrade et al. (2012) also found linoleic acid (84%) as the main fatty acid in feijoa seeds. This study confirmed that linoleic acid is the main fatty acid in feijoa seeds Palmitic acid appeared at 21%, and oleic acid at 19% (Table 3). Lower values were reported for oleic acid (Andrade et al., 2012) in comparison with those found in the present study. Most of the fatty acids (69%) in the seeds were polyunsaturated. There is no information on whether the unsaturated lipids of the feijoa seeds may become bioavailable for humans (Zhu, 2018). Behenic acid that is a cholesterol-raising saturated fatty acid in humans (Cater and Denke, 2001) was also detected in the seeds but in small amounts (0.91%).

**Table 3.** Fatty acids (%) in seeds of feijoa fruit

| Fatty acids | (%) |
|------------|-----|
| Palmitic   | C16:0 | 20.86 |
| Palmitoleic| C16:1 | 0.48  |
| Stearic    | C18:0 | 2.77  |
| Oleic      | C18:1ω9 | 18.57 |
| Linoleic   | C18:2ω6 | 46.23 |
| Linolenic  | C18:3ω9,12,15 | 3.69  |
| Behenic    | C20:0 | 0.91  |

**Essential oil**

The yield of essential oil obtained from feijoa skin of Huatusco and Chapingo were 0.37% ± 0.00 and 0.53% ± 0.00, respectively. Low recovery of essential oil was also obtained in New Zealand (Shaw et al., 1983)
but in Italy have reported until 0.8% (Smeriglio et al., 2019). There were 31 compounds detected, and the most abundant in the fruit of both zones were 3-hexen-1-yl benzoate (15.4%), elixene (9.8%), spathulenol (8.8%), D-germacrene (8.3%), and alpha-cadinol (6.6%) (Table 4). Binder and Flath (1989) reported 3-hexen-1-yl benzoate, elixene and D-germacrene, although not as major components. Two important volatile components of feijoa fruit are methyl benzoate and ethyl benzoate (Smeriglio et al., 2019). These compounds were identified in the present study with less of 1%, and methyl benzoate was not found in the feijoa harvested in the tropical highland. The 3-hexen-1-yl benzoate belongs to the family of benzoic esters like those two compounds, and should play an aromatic role. Caryophyllene (5.3%) (Table 4) was also detected in feijoa fruit cultured in Morocco (Elfarnini et al., 2018), and it is a natural bicyclic sesquiterpene present in many essential oils. Saj et al. (2008), reported that feijoa essential oil has a broad spectrum of antibacterial and antifungal activity mainly due to caryophyllene oxide. Another compound identified in the fruit of both zones was spathulenol that in vitro is potent against the multi-drug-resistant *Mycobacterium tuberculosis* (Dzul-Beh et al., 2019). Selinene was a major component in the feijoa skin in Italy (Smeriglio et al., 2019) but in our work it was not detected.

It was observed that the fruits collected in the tropical highland (Huatusco) had 26 components, and the fruits from the temperate zone (Chapingo) achieved 31. The essential oil recovery was also lower in the fruit skin harvested in the tropical highland.

The type of essential oils that predominated in the skin of the fruit were terpenoids with 71% in the temperate zone (Chapingo), and 69% in the tropical highland (Huatusco). Binder and Flath (1989) reported 53% terpenoids in feijoa skin produced in USA. In both types of fruit zone, the aromatic esters represented about the 19% of the identified components.

**Table 4.** Chemical composition of essential oils of the skin of the feijoa fruit produced in a tropical highland (Huatusco), and in a temperate zone (Chapingo). Calculated retention index (RI), formula, and molecular weight (MW) of compounds are included.

| No. | Compound | IR | Formula | MW | Compound | Chapingo (%) | Huatusco (%) |
|-----|----------|----|---------|----|----------|--------------|--------------|
| 1   | No name  | 986|         |     |          | 0.8          | 0.5          |
| 2   | trans-beta-ocimene | 1037| C10H16 | 136| trans-beta-ocimene | 0.8          |
| 3   | beta-ocimene. | 1048| C10H16 | 136| beta-ocimene. | 0.6          | 0.4          |
| 4   | methyl benzoate | 1097| C8H8O2 | 136| methyl benzoate | 0.4          |
| 5   | Linalool. (M) | 1101| C10H18O | 154| Linalool. (M) | 5.7          | 2.5          |
| 6   | ethyl benzoate | 1174| C9H10O2 | 150| ethyl benzoate | 0.7          | 0.4          |
| 7   | butanoic acid, 4-hexen-1-yl ester | 1187| C10H18O2 | 170| butanoic acid, 4-hexen-1-yl ester | 0.7          | 0.7          |
| 8   | alpha-terpineol | 1198| C10H18O | 154| alpha-terpineol | 0.6          | 0.4          |
| 9   | 2-undecanone | 1294| C11H22O | 170| 2-undecanone | 2.4          | 1.8          |
| 10  | alpha-cubebe (S) | 1351| C15H24 | 204| alpha-cubebe (S) | 1.1          | 0.9          |
| 11  | (-)-beta-bourbonene | 1389| C15H24 | 204| (-)-beta-bourbonene | 0.4          |
| 12  | beta-elemene | 1393| C15H24 | 204| beta-elemene | 1.8          |
| 13  | a-gurjunene | 1413| C15H24 | 204| a-gurjunene | 0.8          |
| 14  | Caryophyllene | 1425| C15H24 | 192| Caryophyllene | 5.3          | 5.3          |
| 15  | 1-butanol 3-methylbenzoate | 1441| C12H16O2 | 204| 1-butanol 3-methylbenzoate | 1.3          |
| 16  | Humulene | 1461| C15H24 | 204| Humulene | 3            | 3.1          |
| 17  | Alloaromadendrene | 1466| C15H24 | 204| Alloaromadendrene | 0.7          | 0.7          |
| 18  | D-germacrene | 1486| C15H24 | 204| D-germacrene | 8.6          | 8.04         |
| 19  | beta-cubene | 1488| C15H24 | 204| beta-cubene | 8.5          |
| 20  | Viridiflorene | 1497| C15H24 | 204| Viridiflorene | 5.5          | 4.5          |
| 21  | Elixene | 1502| C15H24 | 204| Elixene | 8.7          | 10.9         |
| 22  | alpha-farnesene | 1505| C15H24 | 204| alpha-farnesene | 1.3          | 0.2          |
Conclusions

The feijoa fruit dehydrated by a conventional air dehydrator lost 45% of its phenols and flavonoids. Other systems to dehydrate feijoa fruit must be tested. Jam showed the greatest reduction in bioactive compounds. Thus, the feijoa jam processing requires cook novelties to conserve bioactive compounds. The main oil in feijoa seeds was linoleic acid. The main essential oils in the fruit skin were 3-hexen-1-yl benzoate, elixene, and spathulenol. The skin of feijoa, contains essential oils and other chemical properties that requires evaluations in the improving of the human health. The essential oil concentration in the feijoa skin produced in tropical highlands may be lower than in temperate zones. This is the first work showing the essential oils of feijoa produced in Mesoamerica.

Authors' Contributions

JGC-C: author of the article, planning of the research, and data analysis. FF: revision and critical revision, and data analysis. DG-R: preparation of samples in the laboratory and planning of the research. KEG-G, and JMM-H: laboratory data collection, and laboratory analysis. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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|   |     |          |      |          |  |  |
|---|-----|----------|------|----------|--|--|
| 23| 1522| C15H24   | 204  | δ-cadinene| 3.4| 3.6|
| 24| 1526| C15H22   | 202  | trans-calamenene| 2.9| 4|
| 25| 1574| C13H16O2 | 204  | 3-hexen-1-yl benzoate| 17.7| 13.2|
| 26| 1582| C15H24O  | 220  | Spathulenol| 8.6| 9|
| 27| 1589| C14H20O2 | 220  | benzoic acid hept-2-yl ester| 6.7|
| 28| 1592| C15H26O  | 222  | Viridiflorol| 4.05| 5.5|
| 29| 1602| C15H26O  | 222  | Ledol| 2.2|
| 30| 1637| No name  | 1.5  |
| 31| 1659| C15H26O  | 222  | alpha-cadinol| 5.7| 7.5|
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