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Research Article

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.

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From **Volume 49, Issue 1, 2021,** Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal will use article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

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 x 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 fruit: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 $ABTS^{\cdot}$ was prepared by mixing 7mM ABTS with 2.45 mM $K_2S_2O_8$. 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 $ABTS^{\cdot}$ 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 $ABTS^{\cdot}$ 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

Standard errors are shown

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 umaní (*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 quitoense*) 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 be also 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)	1.86 ± 0.14
TF (mg EC g^{-1} FW)	0.47 ± 0.05
ABTS (µmol ET g^{-1} FW)	1.43 ± 0.14
DPPH (µmol ET g^{-1} FW)	7.71 ± 0.56
FRAP (µmol ET g^{-1} FW)	11.75 ± 1.07
Vitamin C (mg g^{-1} DW	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 Morroco (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	986			No name	0.8	**0.5**
2	1037	C10H16	136	trans-beta-ocimene	0.8	
3	1048	C10H16	136	*beta*-ocimene.	0.6	**0.4**
4	1097	C8H8O2	136	methyl benzoate	0.4	
5	1101	C10H18O	154	Linalool. (M)	5.7	**2.5**
6	1174	C9H10O2	150	ethyl benzoate	0.7	**0.4**
7	1187	C10H18O2	170	butanoic acid, 4-hexen-1-yl ester	0.7	**0.7**
8	1198	C10H18O	154	alpha-terpineol	0.6	**0.4**
9	1294	C11H22O	170	2-undecanone	2.4	**1.8**
10	1351	C15H24	204	alpha-cubebene (S)	1.1	**0.9**
11	1389	C15H24	204	(-)-beta-bourbonene		**0.4**
12	1393	C15H24	204	β-elemene	1.8	
13	1413	C15H24	204	α-gurjunene	0.8	
14	1425	C15H24	192	**Caryophyllene**	**5.3**	**5.3**
15	1441	C12H16O2	204	1-butanol 3-methyl-benzoate		**1.3**
16	1461	C15H24	204	Humulene	3	**3.1**
17	1466	C15H24	204	Alloaromadendrene	0.7	**0.7**
18	1486	C15H24	204	**D-germacrene**	**8.6**	**8.04**
19	1488	C15H24	204	β-cubebene	8.5	
20	1497	C15H24	204	Viridiflorene	3.5	**4.5**
21	1502	C15H24	204	**Elixene**	**8.7**	**10.9**
22	1505	C15H24	204	alpha-farnesene	1.3	**0.2**

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	**6.2**
31	1659	C15H26O	222	**alpha-cadinol**	**5.7**	**7.5**

Conclusions

The feijoa fruit dehydrated by a conventional air dehydrator lost 45% of its phenols and flavonoids. Other systems to dehydrated 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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