# Semi-Siccative Oils and Bioactive Fractions Isolated from Reunion Island Fruit Co-Product: Two Case Studies

*Alice Delvar, Pascale de Caro,* Yanis Caro,* Alain Shum Cheong Sing, Rudy Thomas, and Christine Raynaud*

**This paper focused on the use of agro-industrial wastes of strawberry guava (*Psidium cattleianum*) and passion fruit (*Passiflora edulis*) generated by the agricultural industry on Reunion Island, according to two routes: extraction of semi-siccative oils from the seeds and extraction of bioactive compounds from residual pulp and peels. Oil content, fatty acid, carotenoids, tocopherol, and sterol concentrations are determined in the seed oils obtained by four different extraction processes using Soxhlet extraction, extraction by hexane and ethanol, mechanical pressing and supercritical-carbon dioxide (SC-$CO_2$) extraction. The oil extraction yields ranged from 15 to 30% w/w for strawberry guava and passion fruit, respectively. Both oils are classified as semi-siccative and had a similar total unsaturated fatty acid content (88%) with a prevalence of linoleic acid (70–78%). High contents in phytosterols and in α-tocopherol are particularly detected in strawberry guava oil. The antioxidant activities of the bioactive compounds extracted by water and ethanol from pulp and peels are characterized by α,α-diphenyl-β-picrylhydrazyl (DPPH) test. After purification on polymeric resin, significant antioxidant activities are recorded (half maximal inhibitory concentration ($IC_{50}$) from 11 to 50 g $L^{-1}$) and are related to polyphenol contents (20.7 to 42.5 g gallic acid equivalents (GAE)/100 g dry extract).**
***Practical Applications*: There is a great interest on the use of tropical fruit wastes because of their large availabilities. Strawberry guava and passion fruit are often used in the food industry, for juice and jam production. The seeds, pulp, and peel residues are in fact a by-product of their industrial processing, which should be valuably processed instead of just throwing away. We can state that the extracted seed (rich in polyunsaturated fatty acids) of strawberry guava and passion fruit can be used in the industrial production of emulsions, paints, and varnishes. Within the positive aspects of the study, one can also distinguish the use of these tropical fruit by-products as beneficial sources of many valuable bioactive compounds, for example, carotenoids, tocopherols, phytosterols, and especially polyphenols, for functional formulations. Moreover, it may be the experimental basis for further development and use in food industry**

## 1. Introduction

Different industries related to the agriculture sector generate a lot of agro-industrial waste in the form of solid waste (seeds, peels, pulps) or liquid waste. The generated residues are not only biodegradable in nature but often rich in bioactive compounds like functional lipids or polyphenols, depending upon source. Because of their large availabilities and their compositions that could be used in other processes, there is a great interest on the valorization of agro-industrial by-products. The paint and coating industries are one of the larger consumers of solvents in the world, which are mostly derived from petrochemical feedstocks and refinery operations. Regulations on VOC[1] have forced producers to adopt low-solvent and more eco-friendly formulations like water-borne coatings, thermosetting emulsions, colloidal dispersions or two-component systems.[2,3] Effective use of available agro-industrial by-products for production of functional plant-based oil emulsions can contribute to make the coating process cost effective and eco-friendly.

Passion fruit (*Passiflorae edulis* edulis; from the Passifloraceae family), and strawberry guava (*Psidium cattleianum* Sabine;

A. Delvar, Prof. P. de Caro, Dr. C. Raynaud
Laboratoire de Chimie Agro-industrielle (LCA)
Université de Toulouse, INRA
INP-ENSIACET
4 allée Emile Monso, CS 44362
31030 Toulouse Cedex 4, France
E-mail: pascale.decaro@ensiacet.fr

A. Delvar, Dr. R. Thomas
Derivery SAS
4 Rue Léon Talmy, 27290 Pont-Authou, France

Prof. Y. Caro, Dr. A. Shum Cheong Sing
Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments (LCSNSA)
Faculté des Sciences et Technologies
Université de La Réunion
15 avenue René Cassin, 97400 Saint-Denis, Réunion, France
E-mail: yanis.caro@univ-reunion.fr

Prof. Y. Caro
Département Hygiène Sécurité Environnement (HSE)
IUT de La Réunion
Université de La Réunion
40 avenue de Soweto, Terre Sainte
97410 Saint-Pierre, Réunion, France

Dr. C. Raynaud
Centre d'Application et de Traitement des Agroressources
CRT CATAR, ENSIACET
Laboratoire de Chimie Agro-Industrielle (LCA)
UMR1010-INRA/INPT, 4, allée Emile Monso – CS 44362
31030 Toulouse Cedex 4, France

**DOI: 10.1002/ejlt.201800391**

from the Myrtaceae family), are the main tropical fruits produced and transformed by the food industry of the Reunion Island, Indian Ocean. According to the French Department of Agriculture and Food statistics, 500 metric tons of passion fruit and 1130 metric tons of strawberry guava were produced on Reunion island in 2017, on 32 ha and 90 ha, respectively. These two fruits are widely used in the food industry, for juice and jam production (150 metric tons for strawberry guava and 5 metric tons for passion fruit). The processing of these fruits generates large amounts of fruit residues or by-products, in the form of seeds, residual pulp, shells, and peels. The valorization of these tropical fruit by-products is of potential economic and ecological interest for the food industry sector. Biorefinery approaches can be designed to develop new integrated fruit production schemes, including by-product transformation processes. The fruit waste contains several types of bioactive molecules, including functional lipids, polysaccharides, and polyphenols. In the framework of sustainable development, the production of plant extracts containing these compounds from fruit waste should meet the requirements of green chemistry, to limit the environmental pressure on a sensitive island ecosystem.

Many studies have been performed on passion fruit in the last 10 years. The composition of its pulp and its co-products (seeds) has been documented, revealing the presence of polyphenols and flavonoids with potentially useful antioxidant activity.[4,5] Previous studies have described the extraction of oil from passion fruit seeds by conventional methods with hexane,[6] with green solvents,[7] by accelerated solvent extraction[8] or with SC-$CO_2$ assisted by ultrasound.[9] Although previous works were performed for the oil extraction from seeds of passion fruit, no data are available regarding the extraction and characterization of bioactive compounds from strawberry guava (P. *cattleianum*) wastes. About the whole strawberry guava fruit (often collected in regions of Brazil), some studies investigated the volatile constituents,[10] polyphenol content and antioxidant activity.[11] To the best of our knowledge, only Biegelmeyer et al. in 2011[12] and Kobelnik et al. in 2012[13] have reported an oil extraction with hexane from seeds of Brazilian strawberry guava for the elucidation of its fatty acid profile or thermal characterization. Therefore, no data are available concerning the minor organic components like carotenoids, tocopherols, and phytosterols of this seed oil or its physical and chemical properties.

The objective of this study is to find a solution of valuation of the by-products generated by the food industry after extraction of the juice of two tropical fruits from Reunion Island, for example, strawberry guava and passion fruit. Two routes are investigated: the extraction of semi-siccative oils from the seeds and the extraction of bioactive compounds from residual pulp and peels. The work involves the determination of the fatty acid profile, carotenoids, tocopherol, and phytosterol concentrations in the seed oils obtained by conventional (screw pressing and Solid-Liquid extraction) and non conventional (supercritical-carbon dioxide (SC-$CO_2$) processes. The yield and the oil quality (PUFA, acid value, carotenoid, sterol, and tocopherol contents) obtained are compared and discussed to identify processes which could be specifically used for industrial production of paints and varnishes. Through this approach, the feasibility of producing high-value organic extracts using the total collected material (integrated production scheme) is assessed. These drying oils and antioxidant fractions represent local resources adapted to new ecological formulations.

## 2. Experimental Section

### 2.1. Plant Material

The feedstock samples consisted of food industry by-products from purple passion fruit (*Passiflora edulis edulis*) and red strawberry guava (*Psidium cattleianum Sabine*). These residues of fruit juice extraction were collected from local companies on Reunion Island. They consisted of seeds with residual peel and pulp. The moisture contents of the co-products were 22.9% for strawberry guava and 49.1% for passion fruit.

Strawberry guava seeds were washed with water and separated from the residual peel and pulp. The seeds were dried at 60 °C for 48 h. The pulp and peel residues were dried in a convection dryer (CE 130, Gunt Hamburg) at 50 °C with an airflow of 1 m $s^{-1}$, for 48 h. The seeds accounted for 30.6% of the total dry weight of by-products.

Passion fruit co-product (residual pulp and seeds) was dried at 50 °C, with an airflow of 1 m $s^{-1}$, for 48 h. The seeds representing 74.0% of the dry weight were then separated from the rest by sieving through a mesh with 4 mm pores.

### 2.2. Chemical Reagents

Cholestanol, α, δ, and γ-tocopherol, gallic acid, Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), and Wijs reagent were purchased from Sigma Aldrich. Analytical grade solvents were obtained from VWR International.

### 2.3. Seed Oil Extraction

#### *2.3.1. Soxhlet Extraction (Conventional Extraction)*

The dried seeds were ground in a grinder and subjected to Soxhlet extraction. The ground seeds (10 g) were placed in the thimble and 150 ml of hexane was added to the flask. After 4 h under reflux, the solvent was evaporated off, resulting in a yield of 1 to 3 g of oil. The extraction was conducted in triplicate and mean yields were calculated.

#### *2.3.2. Cold Maceration*

Seed oil was extracted by cold maceration in hexane or ethanol. Ground seeds (10 g) were placed into a 250 ml Erlenmeyer flask, to which 200 ml of solvent was added. The medium was stirred continually for 24 h and filtered through grade 1 Whatman filter paper. The solvent was then evaporated off, and 1 to 3 g of oil was recovered.

#### 2.3.3. Mechanical Cold Pressing

Seed oil was extracted with a mechanical screw press (CA 59G, Komet) operating at a maximum oil flow rate of 3–5 kg $h^{-1}$ and powered by an electric motor (1.1 kW). The press cylinder was heated before extraction to a temperature comprised between 80 and 100 °C. The press nozzle had a diameter of 10 mm and the screw speed was 40 rpm. The moisture content of the strawberry guava seeds was adjusted to 12%. The residual oil content of the pressed cakes was determined by Soxhlet extraction.

Mechanical pressing was also performed at laboratory scale, to generate mechanical-pressed oils for physicochemical analyses. The seeds (300 g) were pressed in a screw press (Komet) with a maximum oil flow rate of 1 kg $h^{-1}$, powered by an electric motor (0.75 kW). The press cylinder was heated before extraction to a temperature comprised between 80 and 100 °C. The press had a nozzle diameter of 5 mm, a screw length of 18 cm, and a screw speed of 40 rpm.

#### 2.3.4. Supercritical Dioxide (SC-$CO_2$) Extraction

Extractions were performed in a supercritical fluid unit consisting of a 0.22 L extraction vessel, a 0.22 L separator, a pneumatic pump, and two thermostatic baths. The ground seeds (40 g to 50 g) were loaded into the extractor for each experiment. Temperature, pressure, and $CO_2$ flow rate were kept constant during extraction. The influence of these three variables on oil extraction yield was evaluated. The extraction was stopped when a constant weight was attained.

### 2.4. Seed Oil Characterization

#### 2.4.1. Fatty Acid Analysis

Fatty acid composition was determined by GC-FID, with a capillary column (Agilent, Select FAME, 50 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas, at a flow rate of 1.2 mL/min. The temperature was set to 250 °C for the injector and the detector. The temperature of the oven was maintained at 185 °C for 35 min, then increased to 250 °C by 20 °C $min^{-1}$, and held at 250 °C for 1.75 min. Method for chemical derivation (transesterification of fatty acids to fatty acid methyl esters, FAMEs): 20 mg of seed oil was added to 1 ml of tertio butyl methyl ether. A 100 μl aliquot was then mixed with 50 μl of 0.5 M methanolic trimethyl sulfonium hydroxide. Each sample was analyzed in triplicate. FAMEs were identified by comparison of their retention times with those of pure reference standards, and were quantified using the total areas under the peaks.

#### 2.4.2. Iodine Value, Acid Value, and β-Carotenoids

All analyses were performed in triplicate. For iodine value, 0.15 g of seed oil was dissolved in 20 ml of chloroform, and 25 ml of Wijs solution was added. The mixture was stirred for 30 min in the dark, and 20 ml of potassium iodide (150 g $L^{-1}$) and 100 ml of demineralized water were then added to the Erlenmeyer flask. The iodine value was obtained by titration of this solution with sodium thiosulfate (0.1 mol $L^{-1}$), and is expressed in grams of iodine per 100 g ($g_I$/100 g).

Acid value was determined by titrating 0.2 g of seed oil dissolved in 10 ml of propan-2-ol with potassium hydroxide (0.01 mol $L^{-1}$). Acid value is expressed in milligrams of potassium hydroxide per gram ($mg_{KOH}$/g).

A spectrophotometer (Spectrostar, BMG Labtech) was used to estimate total carotenoid content. The absorbance of a solution prepared by dissolving one gram of seed oil in 2 ml of isooctane was read at 446 nm in a quartz cell with a path length of 1 cm. Total carotenoid content ($W_{\beta c}$) was calculated in milligrams of $\beta$ carotene equivalent per kilogram of oil (mg eq. β carotene/kg), with Equation (1), in which 2610 is the molar extinction coefficient of $\beta$ carotene in isooctane at 446 nm expressed in $g^{-1}$.100 ml $cm^{-1}$, ΔA is the difference in absorbance between the sample and the solvent, I is the optical path length in the cuvette in centimeters, and ρ is the oil concentration in grams of oil per 100 ml of solvent.

$$W_{\beta c} = \frac{1000000 * \Delta A}{2610 * I * \rho} \quad (1)$$

#### 2.4.3. Sterol Analysis

Sterols were analyzed by gas chromatography according to Roche et al.[14] after the extraction of unsaponifiable matter. Oil (150 mg) and cholestanol (100 μg) used as an internal standard were dissolved in 1 M ethanolic potassium hydroxide (2 ml) and heated at 75 °C for 20 min. The solution was cooled, and 1 ml of distilled water and 6 ml of cyclohexane were added. The organic phase was recovered by decantation. A mixture of 160 μl of the unsaponifiable matter extract and 40 μl of methyl imidazole in methyl trimethylsilyl heptafluorobutyramide (50 μl in 1 ml) was heated at 103 °C for 3 min. Samples were analyzed by GC-FID with a CP-Sil 8CB column (Varian, 30 m × 0.25 mm × 0.25 μm film thickness) by on-column injection (volume 1 μl). The carrier gas was helium, at a pressure of 100 kPa. The injector temperature was set to 55 °C for 0.5 min, then increased to 340 °C at a rate of 200 °C $min^{-1}$ and held at 340 °C for 30 min. The detector temperature was set at 365 °C. The oven temperature program was as follows: 160 °C for 0.5 min, increasing to 260 °C at a rate of 20 °C $min^{-1}$, maintenance at this temperature for 5.5 min, followed by an increase to 300 °C at a rate of 2 °C $min^{-1}$, with holding at this temperature for 10 min and a final increase to 350 °C at a rate of 45 °C $min^{-1}$ and maintenance at this temperature for 3 min. The retention times of pure standards analyzed under the same conditions were used for the phytosterol identification. The quantification was performed using the internal standard cholestanol. Phytosterol contents were expressed as mg per 100 g of oil (mg/100 g).

#### 2.4.4. Tocopherol Profile

Tocopherols were analyzed by HPLC (Dionex) with a Kromasil 100 SIL column (250 × 4 mm) and a spectrofluorimetric detector

according to the standard NF EN ISO 9936.[15] The mobile phase consisted of a mixture of isooctane and isopropanol (99.5:0.5v/v), and the flow rate was set at 1.1 ml $min^{-1}$. The excitation and emission wavelengths were 290 and 317 nm, respectively. The samples were prepared by dissolving 10 mg of seed oil in 1 ml of cyclohexane. Analyses (20 µl per injection) were performed in triplicate. Tocopherols were identified by comparison of their retention time with those of external standards analyzed under the same conditions. The quantification was performed by external standardization with standard solutions of α, γ, and δ-tocopherol (1–5 µg $ml^{-1}$). Tocopherol contents were expressed as mg per 100 g of oil (mg/100 g).

### 2.5. Bioactive Compounds Extraction from Pulp and Peel

#### 2.5.1. Extraction

Dried peel and pulp samples were ground in a coffee grinder. Samples were extracted in distilled water or ethanol (96%), with a Fibertec apparatus. The solid:solvent ratio was 1:10. After 1 h, the extracts were filtered and were stored at −20 °C until analysis.

#### 2.5.2. Purification

Polysaccharides were removed with a polymeric resin (Amberlite FPX66, DOW), under the form of particles made of macroreticular aromatic polymer particles (sizes between 0.60 and 0.75 mm and a surface area above 700 $m^2 g^{-1}$). As a pretreatment, 30 g of resin was washed with 120 ml of distilled water, followed by 120 ml of ethanol:water (80:20), and then 200 ml of distilled water. Extract (100 ml in total) was slowly loaded onto the column, which was rinsed with 120 ml of distilled water. The polyphenols-rich fraction was then eluted with 200 ml of ethanol: water (80:20).

### 2.6. Characterization of Plant Extracts

#### 2.6.1. Total Polysaccharide Content

Polysaccharides were analyzed by ion exchange chromatography with a CarboPac PA1 column (Dionex). The eluent was potassium hydroxide, at a flow rate of 1 ml $min^{-1}$. The following concentration gradient was used: 2 mM for 39 min, increasing to 10 mM for 2 min, 100 mM for 8 min and 100 mM for 3 min. The temperature was set to 25 °C. The injection volume was 25 µl. External standards were used for polysaccharide identification. Fucose was used as an internal standard for polysaccharide quantification.

#### 2.6.2. Total Phenolic Content

The total phenolic content of the plant extracts was determined by the Folin Ciocalteu method, as follows. In total, 20 µl of fruit extract, 10 µl of Folin Ciocalteu reagent and 170 µl of sodium carbonate was added to each well of a 96-well microplate. The plate was incubated for 45 min at 45 °C and absorbance was measured at 760 nm with a spectrophotometer (Spectrostar, BMG Labtech). A calibration curve was plotted with a standard solution of gallic acid. Each sample was analyzed in four wells. Results are expressed as grams of gallic acid equivalent per 100 g of sample on a dry basis (GAE/100 g d.b.)

#### 2.6.3. Evaluation of Antioxidant Activity

The antioxidant activity of the extracts was assessed by determining their free radical scavenging activity with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as follows. Extracts were diluted in ethanol to obtain seven different concentrations. A 150 µl aliquot of each sample and 150 µl of ethanolic DPPH solution (90 mg $L^{-1}$) were added to the wells of a 96-well microplate. The plate was kept in the dark for 40 min, with mixing by gentle rotation every 5 min. Absorbance was read at 516 nm with a spectrophotometer (Spectrostar, BMG Labtech). Antioxidant activity was assessed by determining the concentration providing 50% inhibition ($IC_{50}$).

## 3. Results and Discussion

### 3.1. Oil Extraction and Characterization

#### 3.1.1. Oil Content

**Table 1** shows the results obtained for the different oil extraction methods. The highest yields were obtained by cold maceration in hexane: 30% for passion fruit and 15% for strawberry guava, showing a higher potential in extractable oil for passion fruit.

The yield of hexane soxhlet extraction for passion fruit (23.6%) was similar to that reported by Barrales et al.[9] in the same conditions. The oil yield obtained by strawberry guava seed maceration in hexane (15.2%) was slightly higher than that reported by Kobelnik et al.[13] In fact, for strawberry guava, maceration resulted in similar yields for each of the solvents. For passion fruit, the yield obtained by maceration was lower with ethanol than with hexane.

Thermal activation was not required, as maceration at room temperature resulted in the extraction of at least as much oil as Soxhlet extraction. The seed:solvent ratio (1:25) and the contact

**Table 1.** Seed oil extraction yields for (a) Soxhlet extraction for 4 h, (b) maceration at room temperature for 24 h, (c) mechanical pressing with a screw press (CA59G), (d) supercritical $CO_2$ extraction, 100 g $min^{-1}$, 200 bars, 60 °C.

| | | Yield (%) | |
|---|---|---|---|
| Extraction method | Solvent | Strawberry guava | Passion fruit |
| Soxhlet[a] | Hexane | 12.5 ± 1.9 | 23.6 ± 0.9 |
| Maceration[b] | Hexane | 15.2 ± 2.1 | 30.0 ± 1.8 |
| | Ethanol | 15.6 ± 2.0 | 19.9 ± 1.4 |
| Mechanical pressing[c] | | 3.7 | 19.5 |
| SC[d] | $CO_2$ | 9.6 | 21.6 |

time used for maceration (24 h) may have favored better diffusion of the solvent into the ground seeds, thereby improving yield. De Oliveira et al.[7] also reported better yields for solvent maceration than for Soxhlet extraction for passion fruit seeds.

Supercritical $CO_2$ was also used as an extraction solvent. The conditions giving the highest oil yields are presented in Table 1. The impacts of temperature, pressure, and $CO_2$ flowrate on oil extraction yield were studied in preliminary tests. Higher yields were obtained when $CO_2$ flowrate was increased from 50 to 100 g $min^{-1}$ and pressure from 150 to 200 bars. Increasing the temperature from 40 to 60 °C improved yields only for strawberry guava. Thus, optimal parameters (100 g $min^{-1}$, 200 bars, 40 or 60 °C), led to a similar extraction time for strawberry guava and for passion fruit (80 min). This result agrees with the works of De Oliveira et al.[16] and Zahedi et al.,[17] who found respectively 18.5% and 23% yields, under the following conditions: 40 or 60°, 200 or 250 bars, between 1 and 2 h.

The yields obtained by mechanical pressing (Table 1) correspond to a recovering of 23.7% of total seed oil for strawberry guava, and 65% for passion fruit (maceration in hexane being the standard protocol), difference which can be explained by the seeds morphology.

### 3.1.2. Physicochemical Analysis

The physicochemical properties of extracted oils are presented in **Table 2**. Mechanical pressed oils presented similar refractive index. Their iodine values classify them both as semi-siccative oils. For the purposes of comparison, corn oil, and soybean oil (125 and 130 $gI_2$/100 g, [18]) are semi-siccative oils used in food industry.

Acid value is used as an indicator of oil quality, particularly for edible oils. The maximum acid value defined by the Codex Alimentarius Commission[19] is 4.0 mg KOH/g for crude oils. The acid values of all the oil samples tested here were below this limit except for the maceration in ethanol. Strawberry guava seed oil had a free fatty-acid content twice that of passion fruit.

For strawberry guava, all extraction methods yielded oils with similar carotenoid contents. For the extraction conditions tested on passion fruit seeds, the most strongly colored oil was obtained by Soxhlet extraction, with a carotenoid content of 26 mg eq. β carotene/kg. Extracts obtained with SC-$CO_2$ had a lighter color corresponding to the lowest carotenoids content (3.2 mg eq. β carotene/kg). Other authors also found lower carotenoid contents in oils extracted with SC-$CO_2$ from different seeds.[20,21] High carotenoid levels in passion fruit oil may be associated with high levels of antioxidant activity, as reported by Ferreira et al.[6]

**Table 3.** Fatty acid composition of seed oils (% of total fatty acids)

| | Strawberry guava | Passion fruit |
|---|---|---|
| Palmitic acid (C:16–0) | 7.4 ± 0.1 | 9.4 ± 0.1 |
| Stearic acid (C:18–0) | 4.1 ± 0.1 | 2.5 ± 0.1 |
| Oleic acid (C:18–1, n-9) | 9.7 ± 0.2 | 16.6 ± 0.3 |
| Linoleic acid (C:18–2, n-6) | 78.3 ± 0.2 | 70.9 ± 0.5 |
| α-Linolenic acid (C:18–3, n-3) | 0.1 ± 0.0 | 0.4 ± 0.0 |

### 3.1.3. Fatty Acid Composition

The fatty acid profiles of the two seed oils are presented in **Table 3**. The mean values obtained with the various extraction methods are shown, as fatty acid composition was not affected by the extraction process. Both seeds oils were polyunsaturated. The most abundant fatty acid was linoleic acid (C18:2), accounting for 78% of total oil content in strawberry guava and 70% in passion fruit. Passion fruit oil was richer in oleic acid than strawberry guava oil, but the two oils had a similar total unsaturated fatty acid content (88%). The fatty-acid profile of strawberry guava was similar to that reported by Biegelmeyer et al.[12] Extraction method had no effect on fatty acid composition, consistent with the findings of Barrales et al.[9] for passion fruit.

### 3.1.4. Sterol Analysis

The sterol profile of the oils is presented in **Table 4**. Strawberry guava seed oil was richer in sterols than passion fruit oil, with a sterol content of 514 mg/100 g. β-sitosterol was the most abundant phytosterol in both oils, accounting for 45% and 78% of total sterol content for passion fruit and strawberry guava, respectively. Passion fruit oil differed from strawberry guava oil in having higher stigmasterol and campesterol contents, consistent with the findings of Da Silva et al.[22] Strawberry guava oil also contained cycloartenol, which was not present in passion fruit oil.

**Table 2.** Physicochemical properties of extracted seed oils, MP, mechanical-pressed oil (laboratory press); S, Soxhlet extraction; MH, maceration in hexane; ME, maceration in ethanol; SC, supercritical $CO_2$ extraction.

| | Strawberry guava | | | | | Passion fruit | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Extraction method | MP | S | MH | ME | SC | MP | S | MH | ME | SC |
| Refractive index | 1.475 ± 0.001 | nd | nd | nd | nd | 1.474 ± 0.001 | nd | nd | nd | nd |
| Iodine value $g_I$/100g | 139 ± 2 | nd | nd | nd | nd | 136 ± 2 | nd | nd | nd | nd |
| Acid value $mg_{KOH}$/g | 1.8 ± 0.5 | 2.8 ± 0.1 | 2.3 ± 0.2 | 5.7 ± 0.7 | 2.4 ± 0.0 | 0.9 ± 0.1 | 1.7 ± 0.3 | 1.3 ± 0.2 | 7.5 ± 0.5 | 1.1 ± 0.0 |
| Carotenoids mg eq. β carotene/kg | 3.1 ± 0.1 | 3.0 ± 0.1 | 4.3 ± 0.3 | nd | 2.6 ± 0.0 | 8.6 ± 0.0 | 25.9 ± 0.1 | 9.2 ± 0.2 | nd | 3.2 ± 0.2 |
| Color | Yellow | Yellow | Yellow | Orange | Yellow | Yellow-orange | Orange | Yellow-orange | Brown | Yellow |

**Table 4.** Sterol composition of seed oils (mg/100 g)

| | Strawberry guava | Passion fruit |
|---|---|---|
| Campesterol | 17.7 ± 0.2 | 38.7 ± 0.4 |
| Stigmasterol | 8.9 ± 0.4 | 98.6 ± 0.1 |
| β-sitosterol | 400.2 ± 0.6 | 157.5 ± 0.6 |
| Δ5 avenasterol | 46.2 ± 1.8 | 18.5 ± 0.2 |
| Δ7 stigmasterol | 2.6 ± 0.4 | 7.1 ± 1.5 |
| Δ7 avenasterol | 7.5 ± 0.2 | 9.4 ± 0.2 |
| Cycloartenol | 13.8 ± 0.7 | nd |
| Methylene cycloartanol | 14.2 ± 0.7 | 8.9 ± 0.1 |
| Citrostadienol | 3.3 ± 0.3 | 9.6 ± 0.3 |
| Total | 514.4 ± 3.9 | 348.2 ± 3.1 |

### *3.1.5. Tocopherol Analysis*

The results of the tocopherol analysis are shown in **Table 5**. In strawberry guava, the major tocopherol was α-tocopherol, accounting for 60% of total tocopherol content. In passion fruit, γ-tocopherol (64%) predominated, followed by δ- and α-tocopherol (22 and 14%, respectively). Malacrida et al.[23] found that δ-tocopherol was the major tocopherol (56%) in passion fruit oil extracted from seeds dried at room temperature, but they detected no α-tocopherol. These differences may be due to differences in the geographic origin of the fruit or in seed pretreatment. As demonstrated by Lavedrine et al.,[24] seeds dried at 50 or 60 °C and storage may lead to a decrease in tocopherol content.

## 3.2. Pulp and Peel Extracts

### *3.2.1. Extraction and Purification Yields*

As shown in **Table 6**, the extraction yields obtained for hydrophilic molecules were between 11 and 24%. The highest yield was obtained with water for strawberry guava (24.4%). A similar yield was obtained for passion fruit with ethanol (23.6%). Extracts were purified on a polymeric resin (FPX66) known to adsorb polyphenols, to remove polysaccharides in eluent. Aqueous extracts gave higher purification yields than ethanolic extracts.

### *3.2.2. Polysaccharides*

For both fruits, the principal polysaccharides found in extracts were glucose (3.7–22.7 g/100 g d.b.) and fructose (9.5–22.0 g/100 g d.b.). Analyses of the total polysaccharide contents in the crude extracts showed a higher content in polysaccharides for strawberry guava extracts than for passion fruit extracts. In fact, strawberry guava extracts contained 1.5 times and 1.8 times more polysaccharides, respectively in aqueous extracts and ethanolic extracts. A purification step was performed on the extracts to remove the polysaccharides and obtain concentrated fractions of polyphenols with higher antioxidant activity. Analyses on residues of purification confirmed that the polysaccharides removal rate was higher for aqueous extracts. The sugar fraction could be of interest for the food industry.

**Table 5.** Tocopherol composition of seed oils (mg/100 g)

| | Strawberry guava | Passion fruit |
|---|---|---|
| α-tocopherol | 13.7 | 2.5 |
| γ-tocopherol | 6.2 | 11.3 |
| δ-tocopherol | 2.9 | 3.9 |
| Total amount | 22.8 | 17.7 |

**Table 6.** Extraction and purification yields from peel and pulp residues (g/100 g)

| Solvent | | Strawberry guava | Passion fruit |
|---|---|---|---|
| Water | Extraction | 24.4 ± 2.1 | 11.1 ± 3.8 |
| Ethanol | | 17.2 ± 0.9 | 23.6 ± 1.0 |
| Water | Purification | 24.6 | 14.9 |
| Ethanol | | 8.5 | 11.2 |

### *3.2.3. Total Polyphenol Content*

The results of the total polyphenol analyses performed with the Folin-Ciocalteu method are presented in **Figure 1**. This assay is not specific to phenolic compounds as the reagent can be reduced by other chemicals components including carotenoids, sugars, and ascorbic acid.[25] However, this method remains widely used and allows the comparison with existing data in the literature.

Crude peel and pulp extracts from strawberry guava were twice as rich in polyphenols as the corresponding crude extracts from passion fruit, with polyphenol contents ranging from 9 to 10 g GAE/100 g d.b. for strawberry guava, and from 3 to 5 g GAE/100 g d.b. for passion fruit.

The TPC of fresh co-products (0.6 and 2 g GAE/100 g f.w. for passion fruit and strawberry guava, respectively) compared with TPC of these two fresh fruits found in the literature[4,11,12,26,27] indicates that fresh co-products are up to four times richer in polyphenols. Strawberry guava co-products showed higher total phenolic content than several fruits known to be particularly rich in polyphenols like black raspberry, lemon, and grapefruit (670–893 mg GAE/100 g)[28] and could therefore be considered as a new valuable source of phenolic compounds. The high TPC of strawberry guava ethanolic extracts was previously correlated with strong inhibitory effect on α-glucosidase, indicating possible use of these extracts for decreasing blood glucose.[29] In addition to their strong antioxidant activities, phenolic compounds of strawberry guava were also found to have antimicrobial effect and to reduce the survival rates of breast and colon cancer cells,[30] leading to potential application in the pharmaceutical field.

In passion fruit, the main polyphenols were flavonoids, known for their inhibitory action on inflammatory cells.[11,31] Crude extracts containing polyphenols, polysaccharides, and

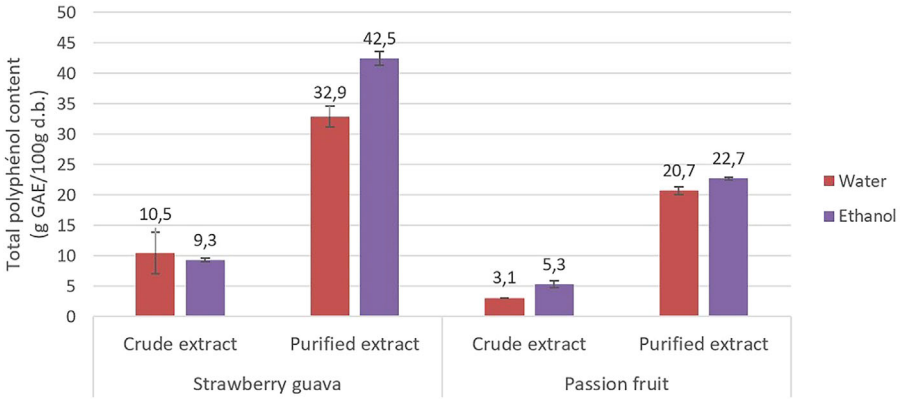


**Figure 1.** Total polyphenol content in crude and purified extracts (g GAE/100 g d.b.).

Vitamin C could also be used for nutraceuticals or food supplements.[32]

Purification resulted in an enrichment of the extracts in polyphenols. Polyphenol contents increased by a factor of 3 to 6.7, reaching a final value of more than 20% in the purified extracts.

Strawberry guava extracts had the highest polyphenol contents after purification (above 30%). Ethanolic extraction resulted in even higher levels (42.5%) of polyphenols in the purified fraction.

However, purified aqueous extracts of passion fruit presented a greater enrichment (multiplication factor: 6.7) in polyphenols than aqueous extracts of strawberry guava, despite their lower polysaccharide contents. In fact, it was shown that the purification step does not only remove polysaccharides but eliminate also the vitamin C, contained in higher amounts in strawberry guava than in passion fruit.[11,33] The purification yielded concentrated fractions of polyphenols that could be useful for possible applications in the pharmaceutical or cosmetic fields.[34]

### *3.2.4. Antioxidant Activity*

The antioxidant activity of extracts was evaluated by determining their free radical scavenging ability with the DPPH assay (**Figure 2**). Crude extracts of strawberry guava had antioxidant activity, with $IC_{50}$ of 37 to 59 mg $L^{-1}$ (the optimal $IC_{50}$ value was obtained with water). These results are consistent with those of Luximon-Ramma et al.,[11] who showed that strawberry guava had the highest levels of antioxidant activity among 17 exotic fruits from Mauritius, including passion fruit. Indeed, the $IC_{50}$ obtained for the strawberry guava co-product ethanolic extract are 10 times lower than the value obtained by Vinholes et al.[29] for fresh fruit ethanolic extract, thus showing the potential of this co-product as a source of natural antioxidants. Crude ethanolic extracts of strawberry guava were found to possess strong in vivo antioxidant properties.[35]

Purification allowed to concentrate extracts in antioxidant molecules by removing the polysaccharides and then to enhance their anti-radical activities. After purification, the aqueous extract of strawberry guava had a high level of antioxidant activity, with an $IC_{50}$ (10–11 mg $L^{-1}$) close to that of Trolox (7 mg $L^{-1}$). Purification was effective, but given the low $IC_{50}$ of crude extracts and the purification yield, the use of crude extracts merits consideration.

The $IC_{50}$ of crude extracts of passion fruit ranged from 300 to 460 mg $L^{-1}$ (the optimal $IC_{50}$ value was obtained with ethanol). The $IC_{50}$ obtained after purification was similar to that for crude extracts of strawberry guava. Purification had a greater effect on $IC_{50}$ for passion fruit extracts than for strawberry guava extracts;

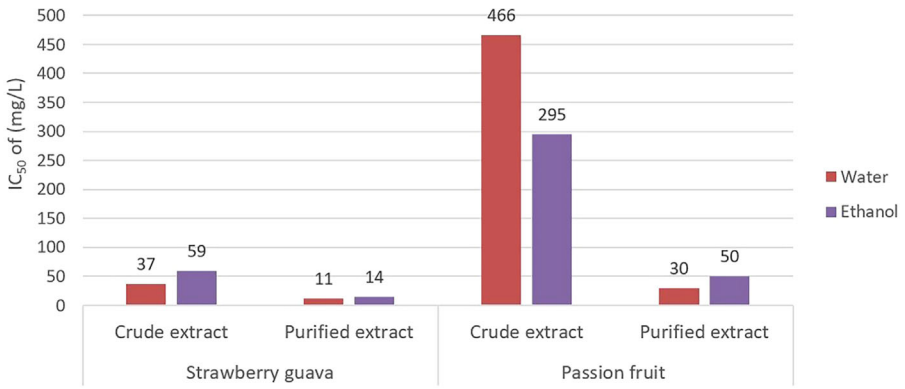


**Figure 2.** $IC_{50}$ of the crude and purified extracts (mg $L^{-1}$).

a multiplication factor of 15.5 was achieved for antioxidant activity enhancement in aqueous extracts of passion fruit. This result is consistent with the high level of polyphenol enrichment observed for the same extract. The activity of flavonoid-enriched fractions of passion fruit suggests possible application in functional food for purified extracts of co-products.[36]

A linear correlation between total polyphenol contents and antioxidant activity of the strawberry guava and passion extracts was highlighted with respective correlation coefficients $R^2 = 0.79$ and 0.92. The higher contents of vitamin C in strawberry guava compared to passion fruit could explain the different correlation coefficients.[4,27] Indeed, previous studies demonstrated that ascorbic acid is included in the total polyphenol content determined by the Folin-Ciocalteu method and provides minor contribution to the antioxidant potential of fruit extracts.[11,25]

## 4. Conclusion

This study indicates that a biorefinery approach can be applied to these two tropical fruit by-products in the food sector. Polyunsaturated vegetable oils can be extracted from the seeds and functional organic molecules can be obtained from the residual peels and pulps. The extraction processes tested here were selected on the basis of their limited impacts on the environment and on health: green solvents (water, ethanol, SC-$CO_2$), moderate temperatures (room temperature or 60 °C), or solvent-free extraction (mechanical pressing).

Oil extraction yields for passion fruit were about twice those for strawberry guava with one of the three solvents (30% and 15%). Ethanol was clearly an efficient solvent for extraction from strawberry guava seeds. Maceration in ethanol was also effective and could easily be scaled up. Moreover, SC-$CO_2$ extraction has the advantage of taking less time and generating oil free of all traces of solvent, despite a yield loss of about 30%. Both plant oils were found to be rich in linoleic acid, with interesting fatty acid profiles (88% unsaturated fatty acids) for use as semi-siccative oils in the coating industry. Moreover, new data were reported on strawberry guava oil composition: high levels of α-tocopherol and sterols, including β-sitosterol in particular. This content in phytosterols combined with a high level in PUFA Omega-6 is interesting for different applications, including food industry. Passion fruit oil is currently used in the cosmetic industry for its antioxidant (mediated by carotenoids) and moisturizing properties.[6]

For extraction from pulp and peel, ethanol tended to give higher yields for both fruits. The extracts obtained had potentially useful polyphenol contents and antioxidant activities, particularly for strawberry guava. In fact, the total polyphenol contents of fresh co-products were found to be up to four times higher than those of fresh fruits. By removing polysaccharides, it is possible to enrich extracts in polyphenols, thereby increasing their antioxidant activity. Strawberry guava extracts enriched in this way had antioxidant activity levels similar to those of Trolox. The fraction of polysaccharides isolated could be of interest to the food industry.

Finally, the bioactive extracts and the seed oils could be combined to formulate innovative plant-based oil emulsions for food or cosmetic fields, but also for ecofriendly painting and coatings with improved preservation properties.

## Abbreviations

FAME, fatty acid methyl ester; GAE, gallic acid equivalent; GC-FID, gas chromatography coupled to flame ionization detector; HPLC, high performance liquid chromatography; IC, inhibition concentration; SC-$CO_2$, supercritical carbon dioxide.


## Acknowledgments

The authors thank Derivery SAS for financial and technical support, as well as National Agency for Research and Technology (ANRT) for funding.


## Conflict of Interest

The authors have declared no conflict of interest.


## Keywords

agro-industrial waste, green extraction processes, passion fruit, strawberry Guava, supercritical $CO_2$



[1] Council Directive 2010 /79/EU of the European Parliament and of the Council of 19 October 2010 on the reduction of VOC emissions *Official J. L304*, 20.11.2010 18.
[2] Y. Lu, R. C. Larock, *Prog. Org. Coat*. **2010**, *69*, 31.
[3] H. Liang, Y. Feng, J. Lu, L. Liu, Z. Yang, Y. Luo, Y. Zhang, C. Zhang, *Ind Crops Prod*. **2018**, *122*, 448.
[4] A. Septembre-Malaterre, G. Stanislas, E. Douraguia, M. P. Gonthier, *Food Chem*. **2016**, *212*, 225.
[5] L. M. Ribeiro da Silva, E. A. Teixeira de Figueiredo, N. M. Ricardo, I. G. Vieira, R. Wilane de Figueiredo, I. M. Brasil, C. L. Gomes, *Food Chem*. **2014**, *143*, 398.
[6] B. S. Ferreira, C. G. de Almeida, L. P. Faza, A. de Almeida, C. G. Diniz, V. L. da Silva, R. M. Grazul, M. Le Hyaric, *Molecules* **2011**, *16*, 5875.
[7] R. C. De Oliveira, S. De Barros, M. Gimenes, *J. Food. Eng*. **2013**, *117*, 458.
[8] G. Piombo, N. Barouh, B. Barea, R. Boulanger, P. Brat, M. Pina, P. Villeneuve, *OCL*. **2006**, *13*, 195.
[9] F. M. Barrales, C. A. Rezende, J. Martinez, *J. Supercritic. Fluids*. **2015**, *104*, 183.
[10] J. A. Pino, R. Marbot, C. Vasquez, *J Agric. Food Chem*. **2001**, *49*, 5883.
[11] A. Luximon-Ramma, T. Bahorun, A. Crozier, *J. Sci. Food Agric*. **2003**, *83*, 496.
[12] R. Biegelmeyer, J. Andrade, A. Aboy, M. Apel, R. Dresch, R. Marin, M. do Carmo Bassols Raseira, A. Henriques, *J. Food Sci*. **2011**, *76*, 991.
[13] M. Kobelnik, D. L. Cassimiro, D. dos Santos Dias, C. A. Ribeiro, M. S. Crespi, *J. Therm. Anal. Calorim*. **2012**, *108*, 1281.
[14] J. Roche, A. Bouniols, Z. Mouloungui, T. Barranco, M. Cerny, *Eur. Lip. Sci. Technol*. **2006**, *108*, 287.

[15] AFNOR, NF EN ISO 9936: Animal and vegetable fats and oils – Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography, **2016**.
[16] R. C. De Oliveira, R. M. Rossi, M. L. Gimenes, S. Jagadevan, W. Machado Giufrida, S. T. Davantel de Barros, *Grasas y aceites* **2013**, *64*, 400.
[17] G. Zahedi, A. Azarpour, *J Supercritic. Fluids*. **2011**, *58*, 40.
[18] S. Naz, H. Sheikh, R. Siddiqi, S. A. Sayeed, *Food Chem*. **2004**, *88*, 253.
[19] Codex Stan.,. Codex Alimentarius, **1999**, Rome, Italy.
[20] L. de França, G. Reber, M. Meireles, N. Machado, G. Brunner, *J. Supercrit. Fluid*. **1999**, *14*, 247.
[21] K. Szentmihalyi, P. Vinkler, B. Lakatos, V. Illes, M. Then, *Bioresour. Technol*. **2002**, *82*, 195.
[22] A. C. Da Silva, N. Jorge, *Food Res. Int*. **2013**, *66*, 493.
[23] C. R. Malacrida, N. Jorge, *Braz. Arch. Biol. Technol*. **2012**, *55*, 127.
[24] F. Lavedrine, A. Revel, A. Poupard, J. Alary, *Food Chem*. **1997**, *58*, 135.
[25] D. Huang, B. Ou, R. Prior, *J Agri Food Chem*. **2005**, *53*, 1841.
[26] M. Isabelle, B. Lee, C. Ong, X. Liu, D. Huang, *Food Chem*. **2010**, *120*, 993.
[27] A. Septembre-Malaterre, G. Stanislas, E. Douraguia, M. P. Gonthier, *Food Chem*. **2016**, *212*, 225.
[28] R. Guimarães, L. Barros, J. Barreira, M. Sousa, A. Carvalho, I. Ferreira, *Food Chem. Toxicol*. **2010**, *48*, 99.
[29] J. Vinholes, G. Lemos, R. Barbieri, R. Franzon, M. Vizzotto, *Food Bioscience* **2017**, *19*, 92.
[30] A. Medina, L. Haas, F. Chaves, M. Salvador, R. Zambiazi, W. da Silva, L. Nora, C. Rombaldi, *Food Chem*. **2010**, *128*, 916.
[31] E. Middleton, C. Kandaswami, *The Flavonoids: Advances in Research since 1986*, (Ed: J. B. Harborne), Chapman and Hall, London **1994**, pp. 619.
[32] S. Dragan, F. Andrica, M. Serban, R. Timar, *Curr. Med. Chem*. **2014**, *22*, 14.
[33] T. Buran, A. Sandhu, Z. Li, C. Rock, W. Yang, L. Gu, *J Food Eng*. **2014**, *128*, 167.
[34] O. Zillich, U. Schweiggert-Weisz, P. Eisner, M. Kersher, *Int. J. Cosmet. Sci*. **2015**, *37*, 455.
[35] J. Cardoso, P. Oliveira, N. Bona, F. Vasconcellos, J. Baldissarelli, M. Vizzotto, M. Soares, V. Ramos, R. Spanevello, C. Lencina, R. Tavares, F. Stefanello, *Redox Rep*. **2017**, *23*, 1.
[36] M. Zeraik, D. Serteyn, G. Deby-Dupont, J. N. Wauters, M. Tits, J. Yariwake, L. Angenot, T. Franck, *Food Chem*. **2011**, *128*, 259.