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# Effects of Temperature and Extraction Time on Avocado Flesh (*Persea americana*) Total Phenolic Yields Using Subcritical Water Extraction

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**Abstract:** This paper investigates the optimum extraction temperature for enhanced total phenolic yields extracted from avocado fruit flesh (*Persea americana*) using subcritical water extraction, as well as the impact of fruit ripeness on phenol extraction efficiency. Additionally, extraction yield against extraction time was investigated for time intervals of 10 min over an overall extraction time of 30 min. The subcritical water conditions studied were 18 bar, 87 mL/min, and temperatures of 105 °C, 120 °C, and 140 °C. The total phenolic compounds content was compared for week one avocado flesh and ripe (week four) avocado flesh, with a four-week ripening period between the two samples. The results show that extracting with subcritical water at 105 °C provides the highest phenolic compounds yields of 0.11% and 0.26% by dried mass for week one and ripe fruit (week four), respectively. The experimental results also indicate that the implementation of lower extraction temperatures on week four avocado (i.e., following the selection of week one avocados and allowing them to ripen over a period of one month) enhances the phenolic compounds extraction yields by more than four times relative to the first week's sample extract, specifically during the first 20 min of extraction.

**Keywords:** avocado flesh; *Persea americana*; subcritical water extraction; total phenolics content; optimized extraction temperature; ripe avocado



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## 1. Introduction

The avocado market has increased drastically in the past decade [1]. This increase appears to be due to avocado oils and bioactive compounds being listed as functional foods that prevent cardiovascular diseases, type II diabetes, cancer, and inflammation [2–4]. Avocado bioactive compounds such as antioxidants, fatty acids, and phenolic components prevent the above-mentioned diseases [2]. In addition to their health benefits, phenols and antioxidants can also be used in the cosmetics industry as hair and skin preserving products. Based on their extraordinary benefits, the phenolic compounds market has been showing an increasing demand over the past years, with a 52% production increase between 1999 and 2013 [1]. In 2015 alone, the phenolic compounds trade exceeded $700 million USD [1]. The growth in consumption of phenols in the therapeutic and cosmetic industries has led to many technical advances in extraction processes to increase extraction yields [1].

For example, traditional methods of extracting avocado oils consist of mechanical extraction by cold pressing or centrifuge procedures. These extraction methods are clean technologies [5]. However, traditional methods are limited by low extraction yields as well as possible sample contamination with the residual debris, the removal of which requires additional filtration methods. More efficient extraction techniques using organic solvents

have been introduced in the market in recent years [5]. Due to their high polarity, pure organic solvents such as ethanol and hexane are more efficient at producing high extraction yields than traditional methods [5]. On the other hand, a major downside to the use of these organic solvents in extraction processes is that traces of these compounds often remain in the extract [6]. This makes the extracted oils less attractive to many consumers, as well as to healthcare and cosmetic industries that must restrict the level of chemical compounds in their products for target markets [7]. Therefore, it is essential to implement innovative methods that use green or natural solvents that do not leave chemical traces in the extract. Promising candidates for these types of solvents are $CO_2$ and water [8,9]. Supercritical $CO_2$ (mixed with 5–10% ethanol as co-solvent) and subcritical water extractions were introduced in the market in recent years [2]. These extraction methods leave minimal to zero traces of the organic solvents, while providing high extraction yields with shorter extraction times [2,10,11].

To give an insight into the extraction type time dependency, Table 1 represents the general extraction time requirements for mechanical press extraction, supercritical $CO_2$ extraction, solvent extraction, and subcritical water extraction. It is shown that subcritical water extraction requires the least time for a complete extraction [2,5,12].

**Table 1.** Extraction time requirements for each of the extraction techniques [2,5,12].

| Extraction Method | Extraction Time |
|---|---|
| Mechanical pressing | 3 h |
| Chemical solvents | 20–24 h |
| Supercritical $CO_2$ extraction | 3–6 h |
| Subcritical water extraction | 0.5–2 h |

Several studies have investigated the extraction yield of avocado bioactive compounds using supercritical and subcritical $CO_2$ extractions at different operating conditions [13]. For instance, Mostert et al. [14] extracted avocado oil using supercritical $CO_2$ at 350 atm and 37 °C with 62.9% oil recovery yield. Although their study showed avocado oil extraction, their extraction quantities are considered to be moderate and have potential for improvement. Botha et al. [15] investigated avocado oil extraction at 540 atm and 81 °C. Their results showed an increase in yield with 94% oil recovery. For a more efficient extraction and less energy consumption during the extraction process, Corzinni et al. [2] conducted an extraction process at 400 atm and 80 °C using ethanol as a co-solvent and were able to achieve 98% avocado oil recovery. Although the above-mentioned supercritical $CO_2$ extraction technique is efficient in extracting avocado oils, the process has not shown high efficiency in extracting polyphenolic compounds. To achieve high extraction efficiencies of polyphenolic compounds, solvents with high selectivity and compatibility with the phenols must be used. Phenolic compounds are polar, and to achieve a high phenols extraction efficiency, a more polar solvent such as water or ethanol should be used [16].

Recent studies have shown that the use of subcritical water and $CO_2$–ethanol mixture extract polyphenolic compounds from natural products in larger quantities than pure supercritical $CO_2$ [13]. For example, Murga et al. [17] proposed the use of supercritical $CO_2$ mixed with polar organic solvent for the purpose of extracting phenolic compounds (coumaric acid, caffeic acid, and ferulic acid) from grape seeds. Similarly, Tian et al. [18] studied resveratrol extraction from grape seeds using subcritical water extraction at 10.2 bar and 152.3 °C. They were able to achieve 6.9 μg/g of resveratrol extract. Another study conducted by Figueroa et al. [16] investigated the phenolic compounds in avocado seed and avocado peel. In their study, Figueroa et al. used a mixture of ethanol and water as a solvent under elevated pressures and temperatures (an extraction method referred to as accelerated solvent extraction). Their results indicated a 15 mg/L extraction rate of antioxidants. Ersan et al. [19] performed subcritical water extraction to extract phenolic compounds from *Pistacia vera* L. Their investigations achieved 22.9 g/kg of gallic acid at 150–170 °C and 69 bar. Tan et al. [20] studied phenolics extraction from avocado using subcritical $CO_2$ and

compared it with ultrasound-assisted aqueous extraction (UAAE) and conventional solvent extraction. The subcritical $CO_2$ extraction ran for 450 min, at 68 bar and 27 °C, whereas the UAAE ran for 30 min, at atmospheric pressure and 35 °C. The solvent extraction method ran for 480 min, at atmospheric pressure and 70 °C. Several studies have investigated the extraction yields of bioactive compounds from natural matrices or their wastes using subcritical water [21]. For example, Karacabey et al. [22] analyzed phenols extraction yields from milled grape canes using a pressurized water extraction with 25% ethanol as a co-solvent. Their results showed an increase in total phenolics by 44% when the ethanol to co-solvent ratio was increased by 25%. Singh et al. [12] reported phenolics extraction yield of 2% (by dried mass) using subcritical water extraction on potato peel. Alvarez et al. [23] also reported 20 mg/g phenolic compounds extraction from potato peel at 40 bar and 190 °C subcritical water extraction. Of all the above extraction techniques, subcritical water extraction showed the highest yields in extracting polyphenolics and antioxidants from phenol-rich biomass. Table 2 summarizes the results obtained from the literature.

**Table 2.** Operating conditions summary.

| Study | Extraction Method | Operating Parameters | Results |
|---|---|---|---|
| Mostert et al. [14] | Supercritical $CO_2$ | 350 atm and 37 °C | 62.9% of avocado oil recovery |
| Botha et al. [15] | Supercritical $CO_2$ | 540 atm and 81 °C | 94% of avocado oil recovery |
| Corzinni et al. [2] | Supercritical $CO_2$ with ethanol | 400 atm and 80 °C | 98% of avocado oil recovery |
| Murga et al. [17] | Supercritical $CO_2$ with polar solvent | Increased pressure from 10 to 250 bar | Phenolics compounds yields from grape seeds increased by 10 times |
| Tian et al. [18] | Subcritical water | 10.2 bar and 152.3 °C | 6.9 mg/kg of resveratrol extract from grape seeds |
| Figueroa et al. [16] | Water and ethanol | | 15 mg/L of antioxidants |
| Ersan et al. [19] | Subcritical water | 69 bar and 170 °C | Extracted 22.9 g/kg of Gallic acid from Pistacia vera L. |
| Tan et al. [20] | Subcritical $CO_2$ | 68 bar and 27 °C | Subcritical $CO_2$ showed 16.97% bioactive compounds yields and UAAE showed 15.13% bioactive compounds yields. |
| Karacabey et al. [22] | Pressurized water with ethanol | Increased ethanol to water ratio by 25% | Total phenolics yields increase by 44% from milled grape canes |
| Singh et al. [15] | Subcritical water | 150–190 °C | 20 mg/g of total phenolics from potato peel |
| Alvarez et al. [23] | Subcritical water | 40 bar and 190 °C | 20 mg/g of total phenolics from potato peel |

A two-factor three-level design of experiment (DOE) was used, with the variation of temperature and extraction time at a fixed flowrate and a fixed pressure. The pressure was chosen according to safe operation requirements representing 50% of the design pressure and based on optimized extraction pressures presented by Khajenoori et al. [24].

Thus, as pharmaceutical and cosmetic industries aim to achieve high levels of phenolics with reduced extraction times using green extraction methods, it is essential to test and apply the subcritical water extraction method. This paper investigates the extraction of phenols from a phenol-rich fruit, *Persea americana*, using subcritical water extraction. This paper also compares extraction yields at three different operating temperatures (105 °C, 120 °C, and 140 °C) over an extraction period of 30 min for week one avocado as well as week four avocado.

## 2. Methodology

To achieve subcritical water state, a pressure and temperature of higher than 1 bar and 100 °C, respectively, is required [25]. The solvent that is injected into the extractor surrounds the solid particles of the avocado flesh. The avocado flesh slices in the extractor experience solvent permeating their surface, which extracts the phenolic compounds. A longer residence time of solvent in the extractor allows the solvent to extract larger amounts of compounds. Due to similar chemical characteristics, the solvent carries with it compounds with similar selectivity and polarity [26]. Following the extractor, the solvent passes through an expansion valve, at which time the high pressure of the solvent is reduced. Extracts and bioactive oils are then cooled down to preserve their properties (i.e., temperatures lower than 10 °C). This is achieved through a condenser, which is placed after the expansion valve to reduce the high temperatures of the solvent/extract mixtures.

The effect of extraction temperature, extraction time, and avocado flesh ripeness were compared. The collected bioactive compounds were analyzed for phenolic compounds count using the Folin–Ciocalteu reagent method [27].

The subcritical water extraction process consists of (i) a 500 mL/min dual piston pump, (ii) a 3 kW electric preheater (Diversified Metal Engineering Ltd. Charlottetown, PE, Canada), (iii) an 8 L stainless steel pressure vessel (Diversified Metal Engineering Ltd. Charlottetown, PE, Canada), and (iv) a shell and tube heat exchanger. Table 3 summarizes the operating conditions of each of the extractions.

**Table 3.** Operating conditions summary.

| Test | Temperature (°C) | Pressure (bar) | Density (kg/m$^3$) | Flowrate (mL/min) |
|---|---|---|---|---|
| 1 | 105 | 18 | 954.74 | 80 |
| 2 | 120 | 18 | 943.08 | 80 |
| 3 | 140 | 18 | 925.90 | 80 |

*Total phenolic compounds determination method*—The collected solvent/extract mixtures were analyzed in duplicate with a total phenolics test using Folin–Ciocalteu reagent and sodium carbonate [27]. For this test, 500 µL of Folin–Ciocalteu and 1.5 mL of sodium carbonate (both reagents were ordered from Fisher Scientific) were mixed with 1 mL of the extract and 6 mL of de-ionized water. In addition to the extract, a set of five standards were prepared using a gallic acid solvent equivalent ordered from Sigma. The reagent reacted with the phenolic compounds in the standards and the extract over a period of 2 h. The reaction changes the color of the phenolics solution, which allows for identification of the phenolics content by spectrophotometer with a wavelength ranging from 200 nm to 830 nm. The spectrophotometer used in this study was manufactured by Eppendorf, Germany.

*Polyphenolics assay*—Using the curve obtained from the gallic acid equivalent, the color variation of the phenolic compounds obtained by the spectrometer in the extract was fitted along the standards curve. The phenolic compounds rate per every liter of water was evaluated using a curve fitting process.

The total phenolics content extracted from the matrix sample was then evaluated using

$$TPC = PC \times SV \quad (1)$$

where $TPC$ is the total phenolic count measured in mg, $PC$ is the phenolic concentration obtained from the total phenolics test measured in mg/L, and $SV$ is the total solvent volume during the extraction process measured in L. The percentage of phenols extracted in comparison to the original sample was evaluated using

$$TPP = \frac{TPC}{SM} \times 100 \quad (2)$$

where *TPP* is the total phenolic extracted percentage measured in %, *TPC* is the total phenolics content measured in mg, and *SM* is the mass of sample in the extractor measured in mg.

In this study, the total phenolics concentrations range is set with the known gallic acid equivalent concentrations using the Eppendorf BioSpectrometer with a UV/Vis spectral range of 200 nm to 830 nm. The calibration curve is prepared using known gallic acid equivalent solution concentrations (see Figure 1). The extracted sample total phenolics concentrations are then evaluated based on the standard curve.

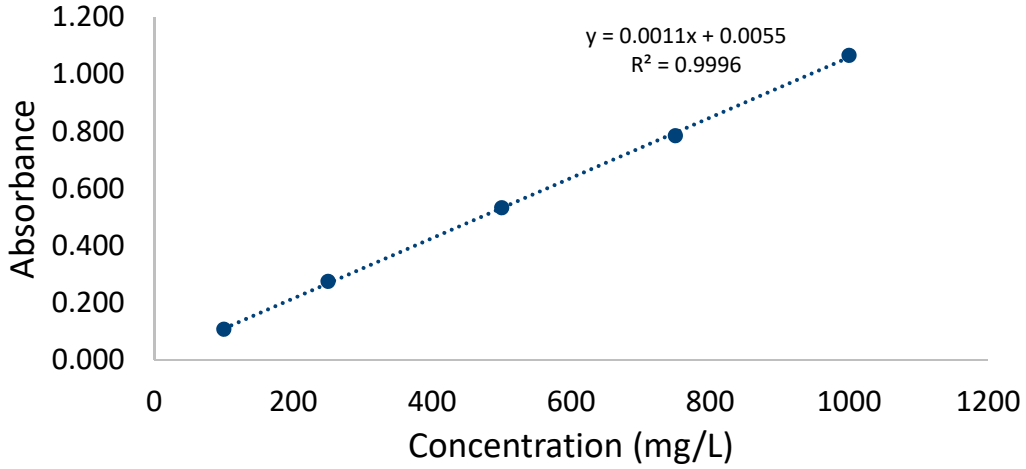


**Figure 1.** Calibration curve of gallic acid.

The extraction yields were measured as

$$Yield = 100 \times \frac{(Total.phenolics.concentration \times Volume.extract)}{Total.substrate.mass} \quad (3)$$

where *Total phenolics* concentration is measured in mg/L, *Volume of extract* is measured in L, and *Total substrate mass* is measured in mg.

## 3. Experimental Setup

*Materials*—A substrate of avocado flesh sample was taken from Hass avocado ordered from the supermarket. Week one Hass avocado was analyzed in two batches differing in ripeness by one month. The batches were identified as first week sample and fourth week sample. In this extraction method, water was transformed from its liquid phase at room temperature and atmospheric pressure to its subcritical phase [28,29], in a continuous extraction (as opposed to a batch extraction). To achieve the subcritical phase, the water was pressurized at 18 bar using temperatures above 100 °C inside an extractor. Subcritical water has increased permeability and selectivity for phenolic compounds from the avocado flesh compared to liquid water at atmospheric pressure and ambient temperature.

The subcritical water extraction process, obtained by BioFoodTech, consists of a pump, preheater, extractor, and condenser (see Figures 2 and 3). Instruments used in the experimental setup include a pressure indicator (PI), temperature indicator (TI), flow transmitter (FT), and a pressure relief valve (PRV). The flowrate used in the extraction system was 87 mL/min. The system used in this study involved open-ended flow. Finally, the extract consisted of phenols mixed with water at the outlet of the condenser.

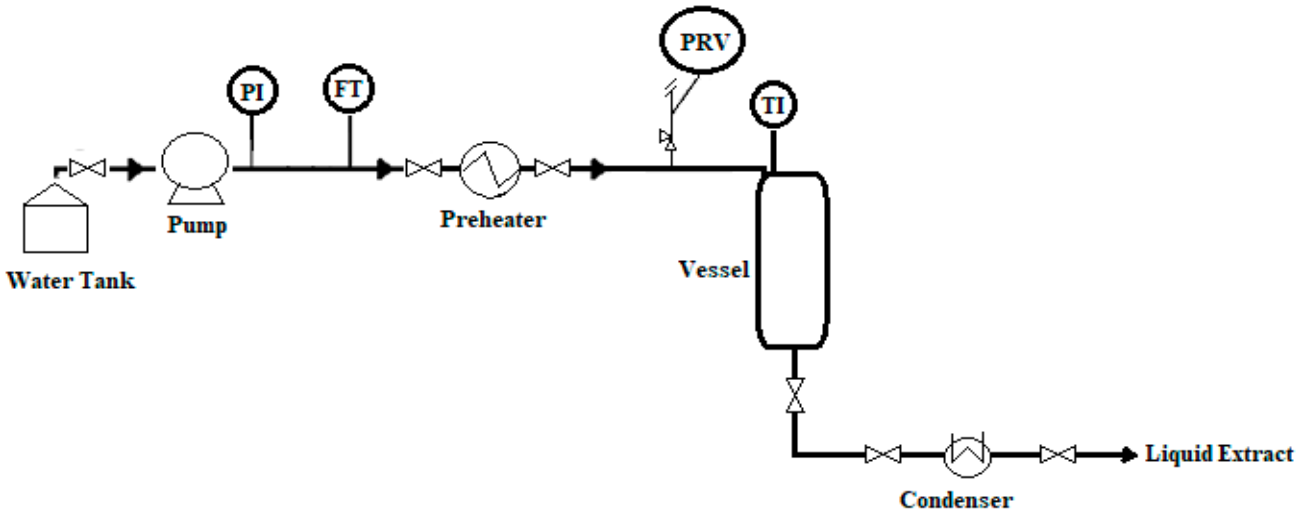


**Figure 2.** Subcritical water extraction flow diagram.


**Figure 3.** Experimental setup of the subcritical water extraction.

## 4. Results and Discussion

The initial wet and dried substrate masses are presented in Table 4. The substrate moisture content was measured to be 83%, and all extracted solutions had a total volume of 2.4 L in 30 min. The total phenolics concentrations presented in Figure 4 and Figure 7 were corrected according to the initial dried substrate masses and moisture content, which are shown in Table 4. The total phenolics yields are calculated using Equation (3).

The extraction rate per water volume for a week one sample at different extraction temperatures is shown in Figure 4. For all temperatures under study (105 °C, 120 °C, and 140 °C), it was observed that the extraction rate per liter of water dropped over the extraction time. This was due to the high water polarity, which adsorbed the highest phenolics amounts during the initial extraction stages. The order of extraction time agreed with previous subcritical water extractions of phenolic components from natural products in terms of phenolic compounds extraction yield reduction over extraction time [30]. The

0–10 min total phenolics values obtained at 120 °C showed higher values than the total phenolics values obtained at 105 °C. This increase was due to the larger volume of the avocado used for that test.

**Table 4.** Substrate mass summary.

| Fruit Condition | Extraction Temperature (°C) | Initial Wet Substrate Mass (g) | Initial Dried Substrate Mass (g) |
|---|---|---|---|
| Week one | 105 | 236.1 | 40.1 |
| | 120 | 479.4 | 81.5 |
| | 140 | 264.9 | 45.0 |
| Week four | 105 | 209.3 | 35.6 |
| | 120 | 240.7 | 40.9 |
| | 140 | 214.6 | 36.5 |

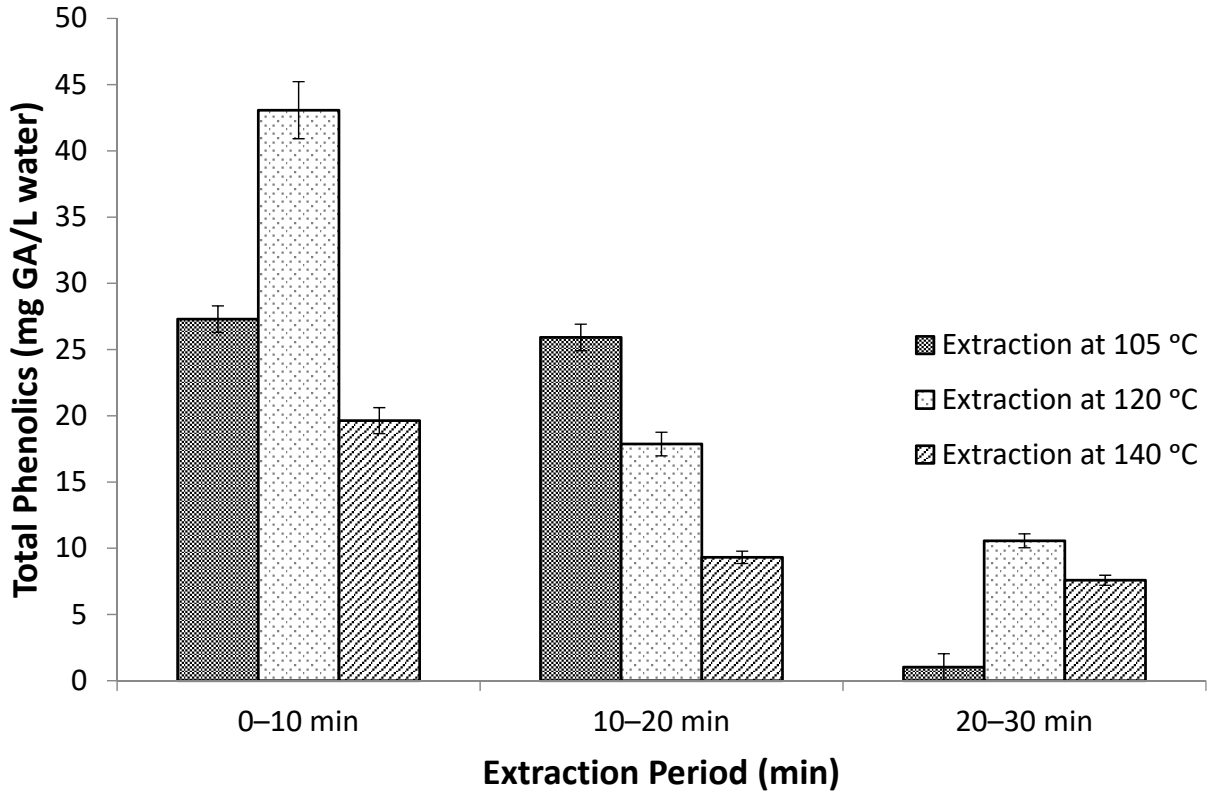


**Figure 4.** Total phenolics extraction for the first week sample.

The extraction yields for a week one sample at different extraction temperatures are presented in Figure 5. It was observed that the extraction yields reduced over the extraction time under all operating temperatures, similar to the observations seen in Figure 4. Figure 5 also indicates that the yields showed the highest values at low extraction temperatures. At 105 °C, water has the highest polarity [10,31] as compared to water at 120 °C and 140 °C, which results in higher extraction of phenolic compounds. The results from this study also showed total phenolics extractions higher than those presented by Figueroa et al. [16] by at least three times. As represented in the Design of Experiment (DOE) analysis and Figures, the variance in the experimental factors was low, indicating a repeatable nature of the experiments. Extraction time also impacted the total phenolics yields obtained.

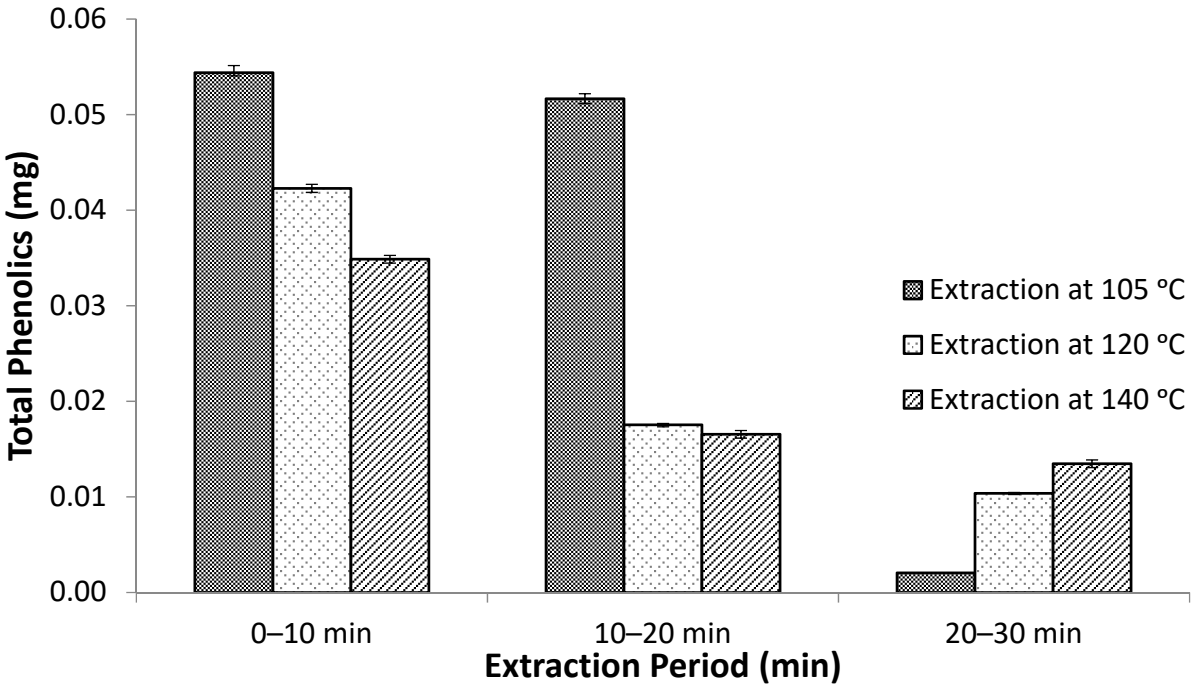


**Figure 5.** Total phenolics yields extracted for the first week sample.

The overall total extraction yields (the sum of all the total phenolics yields at a given temperature over the total extraction period) for a first week avocado sample (i.e., after 30 min of extraction time) at different extraction temperatures are shown in Figure 6. As discussed previously, due to high water polarity at lower temperatures, the extract yields showed the highest values at 105 °C. It was also observed that extraction yields at 120 °C and 140 °C had very close yields to each other. This was due to the exponential reduction of water polarity with increased extraction temperatures [31,32]. In addition to lower water polarity at higher temperatures, high temperatures degrade the quality of the total phenolics if exposed for long periods of time. This could explain another factor that may have led to the reduced total phenolics in each of the tests. Unlike other fruits that require higher extraction temperatures to allow subcritical water to penetrate their thicker surface (such as potato extractions obtained by Alvarez et al. [23]), subcritical water can penetrate the avocado flesh at lower temperatures; hence, it showed higher extraction yields at lower temperatures.

The extraction rate per water volume for a week four (fourth week from the time of purchase) sample at different extraction temperatures is presented in Figure 7. For all temperatures, it was observed that the extraction rate per liter of water dropped over the extraction time. This was due to the high water polarity, which adsorbed the highest phenolics amounts during the initial extraction stages. A combination of the fruit ripeness, which increases the antioxidants and phenols available inside the fruit, along with softer avocado flesh surface may have allowed for increased extraction rates. As per the Villa-Rodriguez et al. [13] study, the phenolic compounds and antioxidants of a fruit increase during the ripening stage due to a burst in ethylene production.

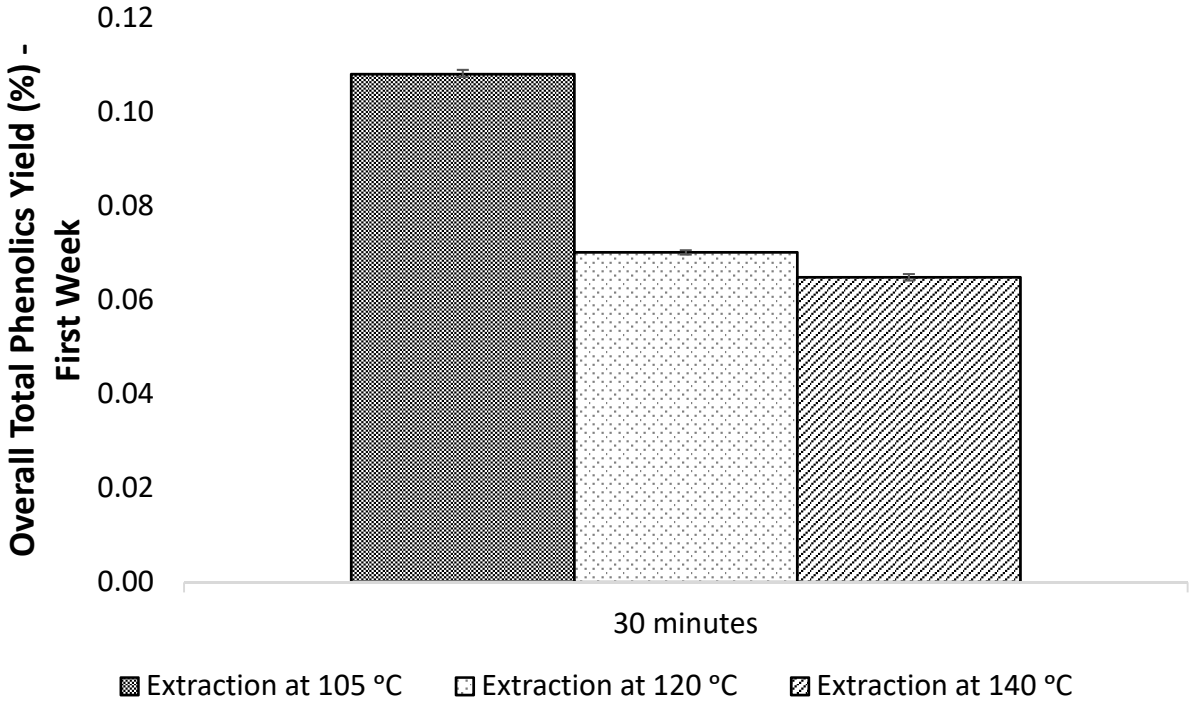


**Figure 6.** Ratios of the overall total phenolics yields extracted for the first week sample.

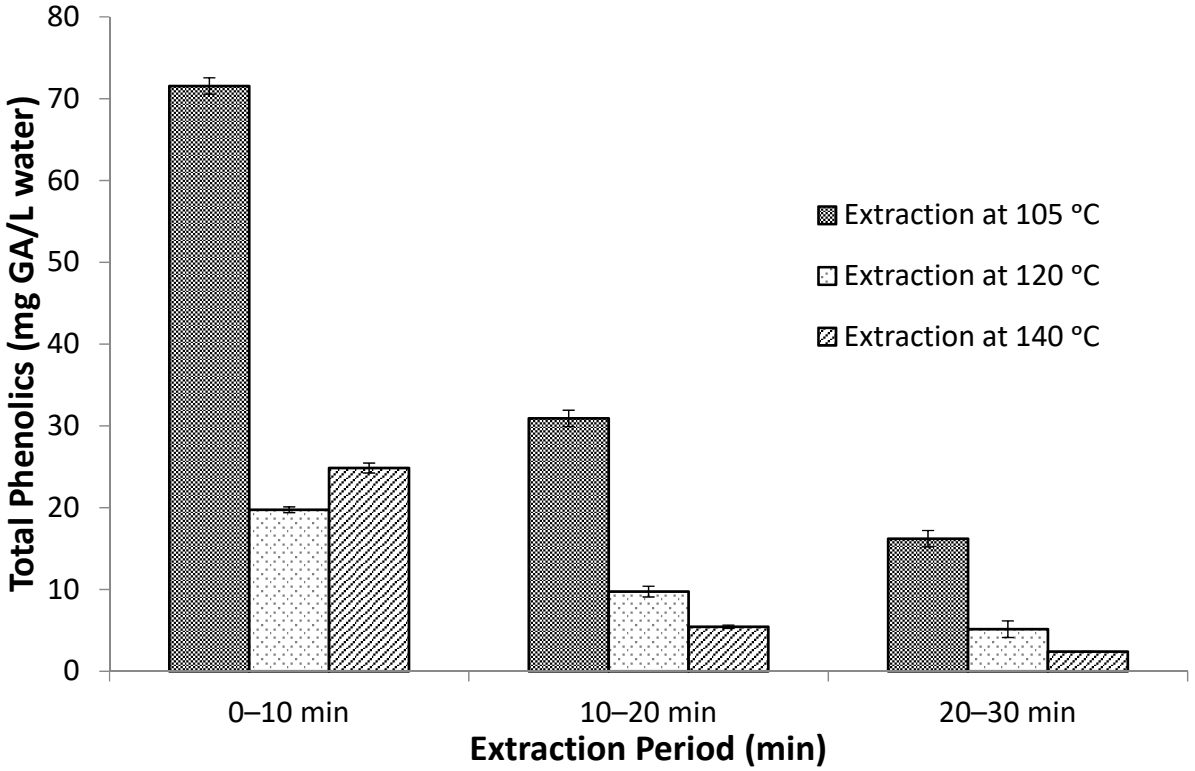


**Figure 7.** Total phenolics extraction for the fourth week sample.

The extraction yields for a fourth week sample at different extraction temperatures is shown in Figure 8; it was observed that the extraction yields dropped with extraction time under all operating temperatures, similar to the results shown in Figure 7. Figure 8. indicates that the yields showed the highest values at low extraction temperatures (around

triple the extraction rates at higher temperatures) due to the higher water polarity and softer avocado surface, which together allow for optimized phenols yield. The extraction yield of the 120 °C sample shows lower values during the first 10 min of extraction as compared to the yields at 140 °C. This could be due to a harder avocado surface during the first 10 min, which prevented the optimized extraction of phenols (as not all avocado samples ripen at the same rate over the surface). As represented in the DOE analysis and Figures, the variance in the experimental factors is low, indicating a repeatable nature of the experiments. The overall total extraction yields (the sum of all the total phenolics yields at a given temperature over the total extraction period) over the 30 min extraction period show very close values between 120 °C and 140 °C, as shown in Figure 9. The week four fruit shows almost four times greater phenol extractions at lower operating temperatures due to the higher water polarity and softer avocado surface.

Extracting the phenolic compounds from avocado shows an extraction rate enhancement from 15 mg/L using the Accelerated Solvent Extraction (ASE) method (applied on avocado seed and peel) [16] to 26 mg/L using subcritical water extraction (applied on avocado flesh). On the other hand, extracting phenolic compounds from avocado shows lower extraction rates when compared to phenolic compounds extractions from potato peel. Hence, avocado waste could be used as an additional source of phenolic compounds from wasted organic materials.

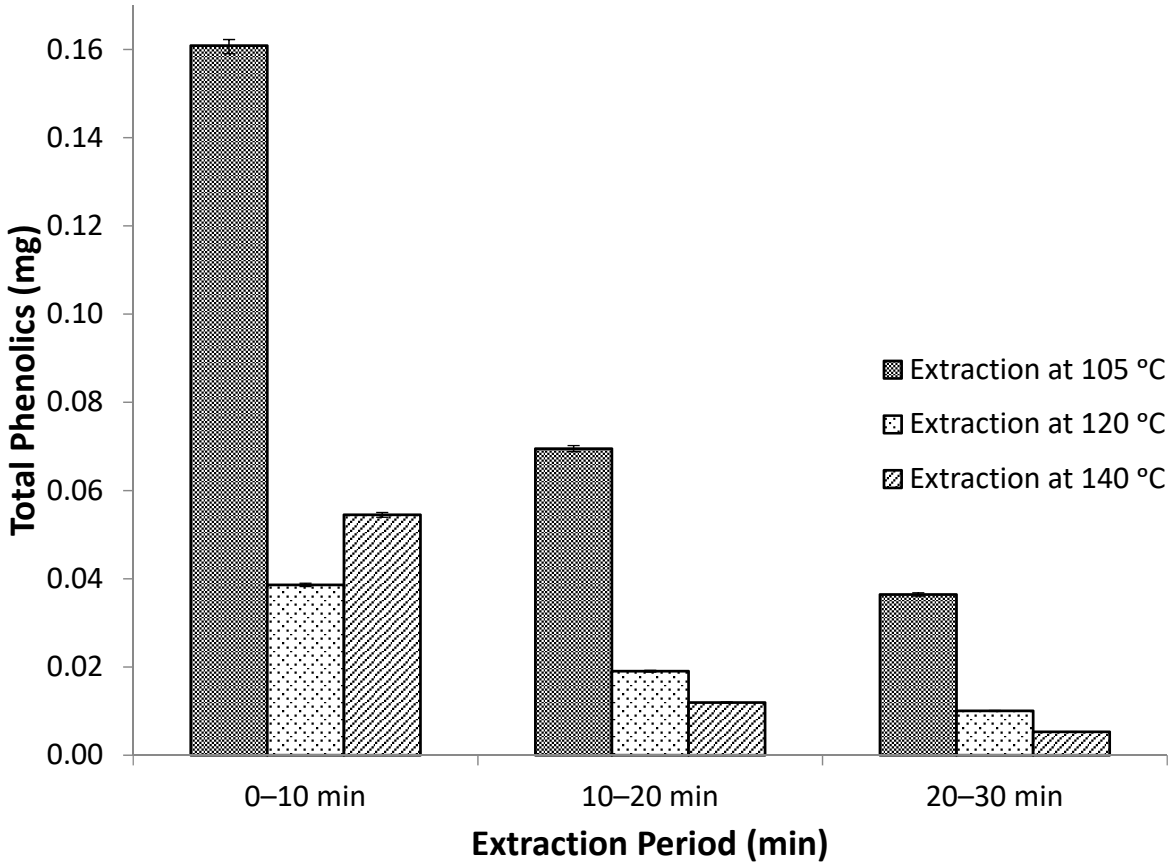


**Figure 8.** Total phenolics yields extracted for the fourth week sample.

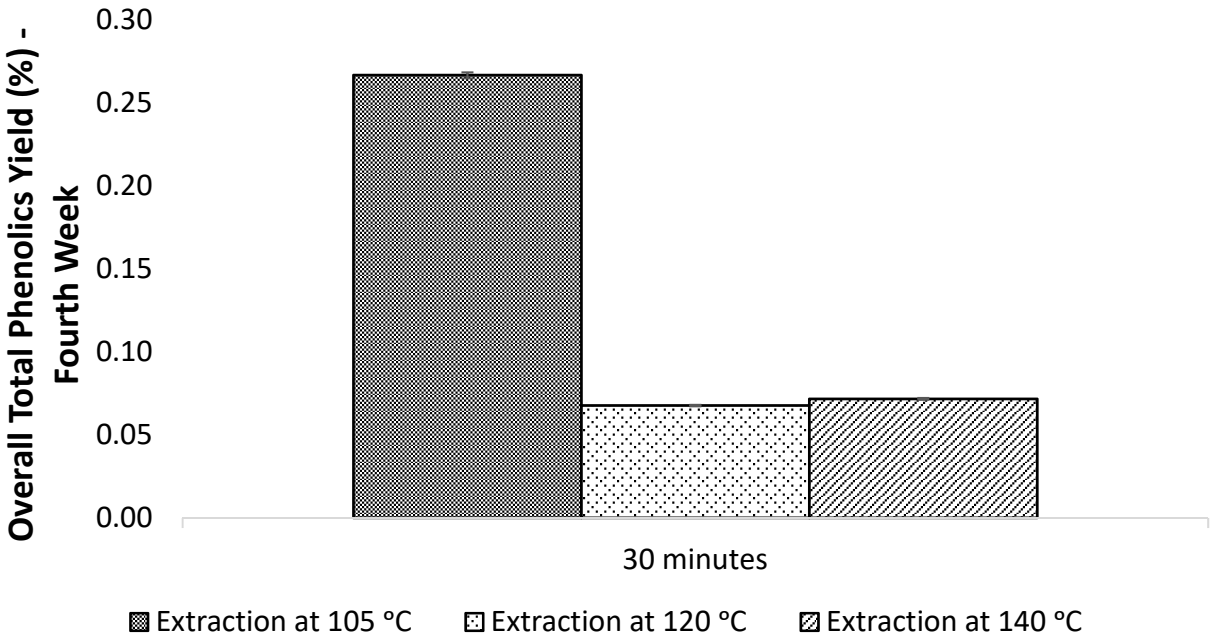


**Figure 9.** Ratios of the overall total phenolics yields extracted for the fourth week sample.

A comparison of the total yield between week one and week four avocado at different temperatures is shown in Figure 10. This figure shows that there is an impact of the fruit ripeness on the total yields at extraction temperatures below 120 °C. On the other hand, Figure 6 shows that there is no impact of the fruit ripeness on the total yields at extraction temperatures above 120 °C. Thereby, it can be concluded that there is no interaction between substrate ripeness and extraction temperatures between 120 °C and 140 °C.

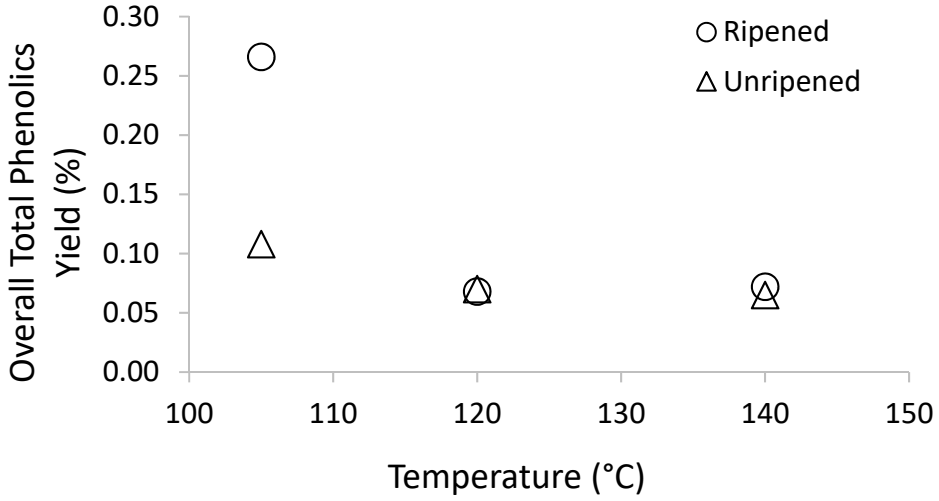


**Figure 10.** Total yields for varying fruit ripeness and extraction temperatures.

Overall, subcritical water extraction is a suitable method that can be utilized to extract phenols from avocado (*Persea americana*). Specifically, extraction at lower operating temperatures of week one avocado present the highest phenols yields of around 0.26% (of avocado dried mass). The highest yields are achieved during the first 20 min of extraction and reduce as the extraction time increases. The results also show very low phenolics yields during the last extraction period. This indicates that 30 min of extraction time is sufficient to extract the phenolic compounds in the avocado flesh.

## 5. Conclusions

The feasibility of using subcritical water extraction to optimize the phenols yields obtained from *Persea americana* is presented. The experimental results indicate that a low extraction temperature of 105 °C is more efficient than higher extraction temperatures (i.e., 120 °C and 140 °C) in obtaining phenolic compounds from avocado flesh. The effect of extraction time is also studied in this paper. For example, the first 10 min during extraction show the highest yields for both week one and week four avocado samples at all operating temperatures. The experimental results also indicate that the implementation of lower extraction temperatures on week four avocado (i.e., following the selection of avocados at week one and allowing them to ripen over a period of one month) enhances the phenolic compounds extraction yields by more than four times relative to the first week's sample extract, specifically during the first 20 min of extraction. Ultimately, the proposed study suggests that subcritical water extraction may be a powerful tool for enhancing phenolic compounds extraction, and that this technology may be of significant value to the healthcare, pharmaceutical, and cosmetic industries.

**Author Contributions:** Conceptualization, W.I.M., A.V., E.C. and A.A.; methodology, W.I.M., E.O., E.M., A.V., E.C. and A.A.; validation, W.I.M., E.O., E.M., A.V., E.C. and A.A.; formal analysis, W.I.M., A.A.; investigation, W.I.M., E.O., E.M., A.V., E.C. and A.A.; resources, E.C. and A.A.; data curation, W.I.M.; writing—original draft preparation, W.I.M.; writing—review and editing, W.I.M., E.O., E.M., A.V., E.C. and A.A.; supervision, E.C. and A.A.; project administration, W.I.M., E.C. and A.A.; funding acquisition, E.C. and A.A. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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