

# Creating Incremental Revenue From Industrial Cherry Wastes

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An increase in the production rate of cherry and cherry products has led to considerable amounts of waste that accumulates rapidly especially during the harvest season. The waste including pits is used as fuel, fertilizer, and partly discarded in the environment. Although the cherry fruit is shown to be a rich source of polyphenols, there is limited information about the polyphenol content of the waste, especially the pits. This study aims to integrate a sequence of novel processing technologies (extraction and encapsulation) for the valorization of cherry pits, i.e. turn them into valuable antioxidant products that can be used in healthcare, cosmetics, and packaging applications. The operative parameters of the extraction process to obtain the maximum polyphenols and encapsulation to protect the extracts were optimized. The results of this study confirmed the feasibility of the extraction and encapsulation to recover a notable concentration of polyphenols with enhanced antioxidant properties. These antioxidants can be used as antibacterial compounds to develop active packaging.

## 1. Introduction

Fruits are an essential source of phenolic compounds with high antioxidant capacity and among all, cherries are of special importance having high phenolic compounds with anti-inflammatory properties (Zhang et al., 2019). Many researchers have been investigating the antioxidant activity of various types of cherries (tart and sour) and their by-products such as cherry pomace through different approaches (de Souza et al., 2014). However, the antioxidant properties of cherry pits have not been investigated yet. Most of the cherries are being processed before selling in the market, which means huge amounts of cherry pits are produced by industry each year. Currently, cherry pits are being used as animal feed, fuel, or preparation of activated carbons, although ultimately most are rendered as waste. However, Cherry pits could be considered a natural source of polyphenolic compounds with antioxidant properties.

Extraction of polyphenols is aimed to recover bioactive compounds such as antioxidants from biomasses. Nowadays there are more attempts to use environmentally friendly extraction methods such as pressure-assisted, ultrasonic, and microwave. These non-conventional extraction techniques could guarantee a sustainable approach resulting in the maximum recovery of bioactive compounds using minimum organic solvent and process cost (Casazza et al., 2010). Among different non-conventional extraction techniques, combined High-Pressure and High-Temperature (HPHT) extraction has gained attention. The synergistic effect of HPHT results in better dissolving and penetration of the solvent thus enhances the solubility of the target compounds in the solvent (Aliakbarian et al., 2011).

Due to antioxidant properties, polyphenols tend to be unstable and their effectiveness and bioactivity dwindle over time during the processing and storage periods (Fang and Bhandari, 2010). Encapsulation is one of the most effective techniques to resolve the instability deficiency by coating the bioactive compound with a physical barrier that can protect the entrapped bioactive compound from environmental conditions such as oxygen, light, and moisture (Rezende et al., 2018). Encapsulation processes used to protect polyphenols generally include spray-drying, freeze-drying, supercritical fluids, emulsions, and chemical methods such as in situ polymerization, and polycondensation (Paini et al., 2015). Among these techniques, spray-drying is the most commonly implemented technique for polyphenols coating (Munin and Edwards-Lévy, 2011). Spray drying is an efficient and economically sustainable technique to convert liquid extracts into a dry powder while protecting active

ingredients with a coating agent. It has been widely used in the food industry. Dry powders are more stable compared to liquid products and are easier for storage and transportation (Paini et al., 2015). Conventional coating agents, such as maltodextrin or gum arabic have been used to enhance the solubility and bioavailability of the product (Ballesteros et al., 2017; Tolun et al., 2016). For this research, maltodextrin and inulin were used as coating agents to enhance the encapsulation properties. Inulin is a well-known compound with functional properties such as prebiotic effects.

To the best of our knowledge, this is the first study focusing on cherry pits valorization as one of the most important by-products of cherry processing. This work aimed to optimize the extraction and encapsulation of phenolic compounds from cherry pits using HPHT extraction and spray drying techniques. The effect of coating agent and inlet temperature were studied. Powders were then characterized for polyphenolic compounds and their antiradical power.

## 2. Materials and methods

### 2.1 Reagents

Folin-Ciocalteu reagent, catechin, sodium carbonate, ethanol (EtOH), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), aluminum chloride, sodium hydroxide, caffeic acid, and gallic acid were purchased from Sigma Aldrich, USA. Cherry pits were kindly provided by a local cherry process company in Traverse City located in Northern Michigan, USA.

### 2.2 Extraction of polyphenolic compounds

Cherry pits were collected from the cherry processor company. Pits were oven-dried for 24 hours at 60 °C. Dried pits were ground to coarse particles using ball mill Spex 8000M. Fine powders were obtained using 20 and 80 mesh sizes. The first batch of experiments was done using conventional solid-liquid extraction at room temperature using two different solvents: 1) 100 % ethanol (EtOH) and 2) water: ethanol EtOH:H<sub>2</sub>O (50:50 v/v). Three different cherry pit particle sizes (420, 841, and 420 < size < 841 μm) were used. The extraction time and the solid to liquid ratio (S/L) were fixed at 24 hours and 0.1 g/mL, respectively (Aliakbarian et al., 2011). The extracts were filtered using a vacuum filter with a 450 nm membrane and the liquid was stored in a close container at 4°C before analysis. Tests on the extracts were performed in the following 14 hours. The best particle size and solvent determined from the conventional extractions were selected for further extraction experiments. Non-conventional extraction tests were performed in HPHT reactor model 4560 (PARR Instrument Company, Moline, IL, USA). The air in the headspace of the reactor was replaced by nitrogen to avoid excess of oxidation during the process and the reactor was hermetically closed. For this step, six runs were performed varying time (30 and 135 minutes) and temperature (100, 125, 150 °C). Extracts were filtered using a vacuum filter with a 450 nm membrane and stored at the close container at 4 °C before analysis. Extract analysis was done in the following 14 hours of the extraction.

### 2.3 Quantification of total polyphenols (TP), total flavonoids (TF), and antiradical power (ARP)

Total polyphenols yield (TP) of the extracts were measured using Folin–Ciocalteu assay (Swain and Hillis, 1959). The extract was filtered using a 0.450 μm PTFE membrane syringe filter (Fisher Scientific Co., USA) before being used for the assay. UV-Vis Spectroscopy (Perkin Elmer) at a wavelength of 725 nm was used. Calibration curves were determined using gallic acid with a linear slope of 0.0018 (mg/L)<sup>-1</sup> and R<sup>2</sup> value of 0.99. TP is expressed as gallic acid equivalent (GAE) weight per weight of dried biomass (DB) (mg GAE/g<sub>DB</sub>). Total flavonoids (TF) were measured according to the colorimetric method developed by Yang et al. (2009) and Aliakbarian et al. (2011) and expressed as milligrams of catechin equivalent (CE) per gram of dried biomass (DB) (mg CE/g<sub>DB</sub>). The extract was filtered using a 0.450 μm PTFE membrane syringe filter (Fisher Scientific Co., USA). The same UV-Vis spectrophotometer was used at a wavelength of 510 nm. The calibration curve for this measurement was established using a standard solution of catechin with predefined concentrations with a linear slope of 0.0016 (mg/L)<sup>-1</sup> and R<sup>2</sup> value of 0.99. Antiradical power (APR) was measured following DPPH assay (Brand-Williams et al., 1995; Sluiter et al., 2008). Original DPPH and sample absorbance were recorded in wavelength of 515 nm using the same UV-Vis spectrophotometer described above. ARP is expressed as gram of DPPH per liter of extract (g<sub>DPPH</sub>/L<sub>extract</sub>).

### 2.4 Encapsulation using spray drying and product characterization

The extract with the highest TP content was filtered using 11 μm pore size filter paper (Fisher Scientific Co., USA) before mixing with the coating agents. Two coating agents maltodextrin (75 g/L) and inulin (75 g/L) were slowly added and mixed into the extracts. Then the solution containing polyphenol extract and coating agent was fed at the rate of 7 mL/min into an Armfield FT80 Tall Form Spray Dryer. The nozzle size was 12 μm. The fluid was dried using hot airflow at two different temperatures (inlet temperature) of 130°C and 150°C. Dry powder was collected from the chamber and stored at 4°C in closed dark vessels before analysis. Post spray drying, a portion of the powder was immediately separated and rheumatically sealed until ready for water activity

measurement. The water activity of the spray-dried samples was measured using an Aqualab 4TE (Meter Group, Pullman, WA, USA). Once ready, the samples were placed in the calibrated water activity meter for recording. The Aqualab 4TE is a child mirror dew point sensor that allows for a  $\pm 0.003$  accuracy of the measurement. Samples were taken after 14 and 21 days of storage at 4°C and the TP and TF were measured.

### 2.5 Statistical analysis

Statistical analysis was performed using MATLAB and SAS using a multivariate analysis of variance. All samples were tested in three replicates and the average and standard deviation were determined. Results were compared statistically using a two factorial ANOVA table by MATLAB software and  $p$ -value  $\leq 0.05$  was considered as a statistically significant difference.

## 3. Results and discussion

### 3.1 Effect of particle size and solvent on TP content

Table 1 shows the TP yield of cherry pits extracts using conventional extraction at room temperature. Based on the statistical analysis at 0.05 confidence interval, the TP means are all significantly different when different particle sizes were used. Ground pits with a smaller particle size (420  $\mu\text{m}$ ) showed significantly higher TP yield. When particles were additionally ground from 840  $\mu\text{m}$  to 420  $\mu\text{m}$ , TP yield was increased by 136 % and 216 % for EtOH: H<sub>2</sub>O and EtOH extraction, respectively. The smaller the particles, the more surface area (per unit mass) is exposed to extracting solution which results in higher polyphenols yield. Also, as for smaller particles the solute can more easily diffuse through the solid matrix which ultimately increases the TP yield (Gião et al., 2009). Similar results were found in the literature regarding the extraction of polyphenols from different biomasses (Casazza et al., 2010; Makanjuola, 2017).

Table 1. TP yield of ground cherry pits at different particle sizes and solvents. For each solvent treatment, means in a column followed by different letters (from a to c) are significantly different at  $p < 0.05$ .

Particle Size: x ( $\mu\text{m}$ )	100 % EtOH extraction TP Yield (mg GAE/g <sub>DB</sub> )	EtOH:H <sub>2</sub> O (50:50 v/v) extraction TP Yield (mg GAE/g <sub>DB</sub> )
x<420	2.56 $\pm$ 0.05 <sup>a</sup>	3.57 $\pm$ 0.05 <sup>a</sup>
420<x<840	2.38 $\pm$ 0.05 <sup>b</sup>	2.84 $\pm$ 0.01 <sup>b</sup>
840<x	0.82 $\pm$ 0.01 <sup>c</sup>	1.51 $\pm$ 0.06 <sup>c</sup>

The choice of the extracting solvent could significantly affect the polyphenols yield (Kashif et al., 2017). Solvents that could achieve faster diffusion, increase extraction efficiency. Also, low toxicity, potential environmental issues, and the cost of the solvent are among the factors to be considered (Kashif et al., 2017). The most common solvents used for the extraction of phenolic compounds from cherry by-products are methanol (Adil et al., 2008), ethanol (Woźniak et al., 2016), 2-propanol (Rødtjer et al., 2006), acetone (Greiby et al., 2017) either pure or diluted in water. Controversial results have been reported since some studies have shown that using absolute organic solvents resulted in a higher yield of polyphenols compared to the diluted solvent (Thouri et al., 2017), while other studies showed higher yield when using a diluted solvent (Ćujić et al., 2016). In a study by Yilmaz et al. (2015), it was shown that the TP yield of sour cherry pomace was lower at low EtOH concentration, reaching the maximum yield of 14.23 mg/g at an ethanol concentration of 51 %. Some polyphenols tend to be more soluble in organic solvents while some are more soluble in inorganic solvents such as water. Extracts using a mixture of water and ethanol (1:1 v/v) have shown significantly higher TP yield compared to the extracts using only ethanol as solvent. Similar results were obtained by Ćujić et al. (2016) who has shown that 50 vol% ethanol solvent was the optimum extraction solvent when extracting polyphenols from chokeberry dried fruit. The particle size of 420  $\mu\text{m}$  and EtOH:H<sub>2</sub>O (50:50) were selected for further experiments.

### 3.2 Effect of High Pressure-High Temperature (HPHT) extraction on the content of TP, TF, ARP

Extraction at high pressure and high temperatures above the solvent's boiling point is shown to improve the extraction yield of polyphenols. At elevated temperatures, mass transfer rate and solubility properties are enhanced resulting in more phenolic compounds dissolving into the solvent (Arwa and Turner, 2011). At high pressures, more solvents can penetrate the solid matrix and extract more phenolics (Carabias-Martínez et al., 2005). However, prolonged exposure of phenolic compounds to high temperatures could potentially lead to the degradation and oxidation of thermosensitive compounds (Casazza et al., 2010).

**Errore. L'origine riferimento non è stata trovata.**..a presents the effect of extraction time and temperature on the total yield of polyphenols. At lower temperatures, the contact time seems to have a greater impact on TP yield. At 100 °C extending the extraction time from 30 to 135 minutes almost doubled the TP yield, however, at higher temperatures i.e. 125 and 150 °C, the extension of extraction time increased TP yield by only 35 % and 21 %, respectively. At shorter extraction times, the temperature's impact is greater. For instance, at extraction

time of 30 minutes elevating the temperature from 100 ° C to 150 ° C increased TP yields 3.2 times, however at extraction time of 135 minutes, elevating temperature (from 100 ° C to 150 ° C) only doubled TP yield.

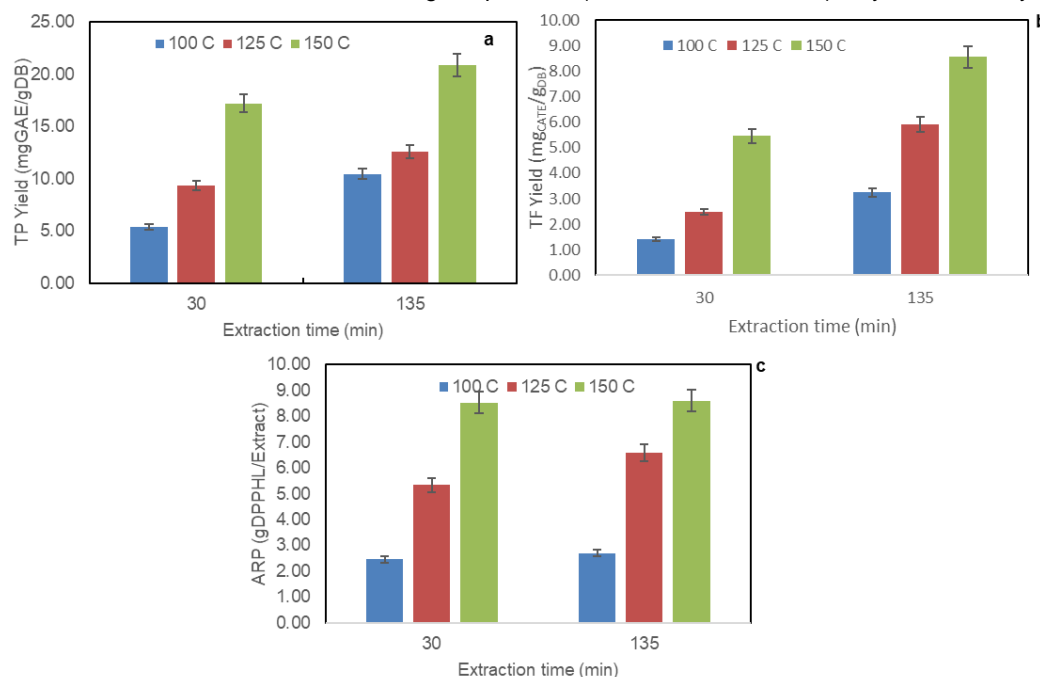


Figure 1. Properties of cherry pits at different extraction times and temperatures: a) total polyphenols yield (TP), b) total flavonoids yield (TF), and c) antiradical power (ARP).

**Errore. L'origine riferimento non è stata trovata.** b shows the effect of extraction time and temperature on total flavonoid contents. A very similar trend to TP was noticed. Higher extraction temperature resulted in higher TF content. After 30 minutes of extraction, TF increased from 1.45 (mgCE/g<sub>DB</sub>) to 7.30 (mgCE/g<sub>DB</sub>). At a longer extraction time of 135 minutes, TF content resulted in to increase of 2.6 folds (from 3.77 mgCE/g<sub>DB</sub> to 10.06 mgCE/g<sub>DB</sub>) when the temperature was increased from 100 ° C to 150 ° C.

The Antiradical Power (ARP) of the extracts at different extraction times and temperatures are illustrated in Figure 1.c. There seems to be a strong correlation between the total polyphenols yield and the antiradical activity of the extract. The highest ARP was achieved at 30 minutes and 150 ° C (8.53 g<sub>DPPH</sub>/L<sub>extract</sub>). A longer extraction time did not enhance the ARP. Antiradical Power was 8.60 (g<sub>DPPH</sub>/L<sub>extract</sub>) at 135 minutes and 150 ° C.

### 3.3 Effect of spray-drying on the stability of samples

The cherry pit HPHT extract obtained at 30 minutes and 150 ° C was spray-dried using maltodextrin (M) and inulin (I) at two inlet temperatures (IT) of 130 ° C and 150 ° C. Powders from the spray dryer were tested for water activity and the recovery yield was also measured (Table 1). Aliquots of samples were stored at 4 ° C for 14 and 21 days and their TP and TF were determined.

Table 2. Water activity and recovery of spray-dried samples.

Sample	Inlet Temperature (°C)	Covering Agent	Powder Recovery (%)	Water Activity
M130	130	Maltodextrin	66.5	0.2664
I130	130	Inulin	51	0.1943
M150	150	Maltodextrin	66.1	0.2224
I150	150	Inulin	70.6	0.1172

As shown in Table 2, when inulin was used as the coating agent, and IT was fixed at 150 ° C powder recovery was the highest (70.6 %) and water activity was the lowest (0.1172). IT did not show any significant effect on the recovery percentage and the water activity of powders when maltodextrin was used. In both inlet temperatures, inulin resulted in power with the lower water activity compared to the maltodextrin. Water activity is an important factor affecting the stability of powders and dehydrated products during storage.

To determine the stability of the spray-dried samples, powders and the extract were stored at 4 °C for 21 days. Table 3 shows the effect of storage time on TP and TF of the powders compared to the initial extract. TP content of the initial extract was not changed after 21 days stored in a fridge at 4°C. Spray-dried powders were stored in the same condition. The TP content of all samples was unchanged after 21 days. However, the total flavonoid content of the samples showed dramatic changes over this period. This is because flavonoids are more sensitive to environmental parameters such as exposure to oxygen, light, and they degrade faster than other types of polyphenols. From day 14 to day 21, the extract had lost almost 30% of its flavonoids content, while encapsulated samples with maltodextrin were statistically unchanged, and encapsulated samples with inulin had only 11-16 % decrease in TF. It seems safe to assert that encapsulation has protected flavonoids for at least 21 days since no drastic change was observed in encapsulated samples compared to the initial extract.

*Table 3. Effect of storage time on TP, TF, ARP on spray-dried samples*

Sample	Storage Period (days)	TP (mg GAE/g)	TF (mg CE/g)
Extract	14	16.4	2.1
Extract	21	16.5	1.5
M130	14	16.1	1.9
M130	21	18.0	1.9
I130	14	16.2	1.9
I130	21	17.6	1.6
M150	14	16.5	1.9
M150	21	18.4	1.8
I150	14	15.7	1.8
I150	21	17.3	1.6

#### 4. Conclusions

This study shows that there is a potential to isolate high-value products from cherry pits using green and environmentally friendly processes; the extraction was performed using Generally Recognized as Safe (GRAS) solvents aiming to reduce the wastes generated from the cherry process. The effect of particle size, solvent, extraction time, and temperature was studied on total polyphenols yield (TP), total flavonoids yield (TF), and antiradical power (ARP) of cherry pit extract. It was found that decreasing the particle size from 840 µm to 420 µm increased the TP by at least 136% in room temperature extraction. Also, a 50:50 v/v mixture of ethanol and water resulted in higher TP and TF by at least 40% compared to pure ethanol extraction. This effect was more severe as the particle size became smaller. Extraction at high pressure and high temperature in the absence of oxygen showed to increase in TP, TF, and ARP. Extending the extraction time to 135 minutes and temperature to 150°C resulted in the highest TP, TF, and ARP with values of 21.47±0.8 mg GAE/g<sub>DB</sub>, 10.06±0.21 mg CE/g<sub>DB</sub>, and 8.6 g<sub>DPPH</sub>/L<sub>extract</sub>, respectively. Spray-drying method using maltodextrin and inulin as coating agents showed to preserve the flavonoids over the 3 weeks testing period while uncoated liquid extract which was stored in a dark near-freezing temperature, lost nearly 30% of their flavonoids. More experiments are under investigation to study the antibacterial properties of cherry pit extracts and encapsulated powders. The polyphenolic compounds with antiradical power could be used in different applications such as anti-aging creams, active packaging, or functional foods. The production of value-added products from cherry pits results in a value-based model for a business entry plan generating profits for the cherry producers.

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