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Comparative Study on Conventional and Microwave-Assisted Ethanol Extraction of Phenolics and Flavonoids from Waste Onion Leaves

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ABSTRACT

This study explored the utilization of waste onion leaves for the extraction of important bioactive compounds known for their health benefits such as phenolics and flavonoids. Conventional solvent (CSE) and microwave extraction (MAE) of flavonoids and phenolics from waste onion leaves using aqueous ethanol as solvent were performed. The effects of different factors such as ethanol concentration (50 %v/v & 80 %v/v), temperature (60 °C & 80 °C for CSE) or power level (10 % & 50 % for MAE), and extraction time (1 hr & 4 hrs for CSE, 10 sec & 60 sec for MAE) on the total flavonoid yield (TFY) yield and total phenolic yield (TPY) were evaluated via a two-level factorial experiment. Results showed that for CSE, ethanol concentration, extraction time and the interactions of the factors significantly affected the TFY ($p \leq 0.05$), while all the three factors, ethanol concentration-temperature interaction and ethanol concentration-extraction time interaction significantly affected the TPY ($p \leq 0.05$). On the other hand, for MAE, ethanol concentration and power level have significant effects on the TFY ($p \leq 0.05$) while the ethanol concentration significantly affected the TPY ($p \leq 0.05$). The maximum extractable flavonoid and phenolic yields from the onion leaves were found to be 8.61 mg quercetin equivalent (QE)/g dry weight (DW) and 8.95 mg gallic acid equivalent (GAE) /g DW, respectively. For CSE, high levels of both ethanol concentration (80 %v/v) and low level of extraction time (1 hr) resulted in higher values of TFY (2.63 mg QE/g DW) and TPY (6.15 mg GAE/g DW). For MAE, higher values of TFY (2.98 mg QE/g DW) and TPY (4.24 mg GAE/g DW) were obtained at the high level of ethanol concentration (80 %v/v ethanol), high power level (50 %), and high level of extraction time (50 sec). MAE was found to be a more advantageous extraction method than the conventional one, because of its comparable flavonoids and phenolics recovery at a shorter extraction time.

Keywords: onion leaves, flavonoids, phenolics, extraction, ethanol

INTRODUCTION

Onions (*Allium cepa* L.) are one of the most indispensable spices in the Filipino cuisine, with an approximate global annual production of around 66–85.7 million tons (Ren et al., 2020). In the Philippines, 124,170 metric tons of onions were produced from January to March of 2018 (Philippine Statistics Authority, 2018). According to Sagar et al. (2018), approximately 25 % to 30 % of many fruit and vegetable farming are generated as waste and for onions, the wastes are mainly in the form of its outer leaves, peel, and skin, which are either discarded or burned by local farmers. However, these disposal methods may further contribute to waste pollution and ozone depletion while composting of these wastes cannot be done because of the pungent smell which promotes rapid growth of phytopathogenic microorganisms (Breu, 1996). Instead, several studies have pointed out that these agricultural wastes can be further utilized by extracting high value products such as pigments, phenolic compounds, dietary fibers, sugar derivatives, organic acids, minerals, etc. that can be used in food sectors, pharmaceuticals, healthcare sectors, and chemical industries (Sagar et al., 2018).

One of the most abundant bioactive compounds in onions is a class of phenolic compounds called flavonoids. Out of its subclasses, flavonols compose the majority; and 80 % to 93 % of the total flavonol content in onions is reportedly made up of quercetin and quercetin glucosides (Lee et al., 2014; Lombard et al., 2000; Price & Rhodes, 1997; Slimestad et al., 2007). Quercetin is a well-studied compound because of its numerous health benefits such as antioxidant, anti-inflammatory, antimicrobial, anticancer antihistamine, anti-edematous, and anti-aging properties (Dmitrienko et al., 2012; Leighton et al., 1992; Shi et al., 2016; Yoshida et al., 1990). Though most studies focus on the fleshy bulb of onions, several studies also suggest that onion wastes also contain valuable bioactive compounds (Miean & Mohamed, 2001; Sagar et al., 2018). Shi et al. (2016) mentioned that the onion skin has a larger quantity of dietary flavonoids compared to the fleshy bulb. In another study, Slimestad et al. (2007) stated that the antioxidant activity is higher in the outer scales than the inner scales of onions,

implying that the outer scales have higher amounts of flavonoids. Also, El-Hadidy et al. (2014) found that the leaves of both Giza 6 and Photon spring onions in Egypt have higher total flavonoids and phenols than in the bulbs. Moreover, Miean and Mohamed (2001) conducted a study on the flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of 62 edible tropical plants including onion leaves, papaya shoots, broccoli, carrot, etc. in which onion leaves had the highest total flavonoids (1497.5 mg/kg quercetin, 391.0 mg/kg luteolin, and 832.0 mg/kg kaempferol).

To obtain phenolic compounds from plant materials, solvent extraction is the most commonly used method due to its accessibility, efficiency, and versatility (Stalikas, 2007). Conventional solvent extraction (CSE) is one of the traditional methods of solvent extraction; hence, it is commonly used as a basis of comparison with other methods. It may produce a lower recovery than the more advanced techniques; but it is usually cheaper and simpler (Sagar et al., 2018). According to Ren et al. (2020), the other extraction techniques that have been employed in the extraction of flavonoids from onions and its wastes (skin, trimmings, non-edible part of the onion bulb that is the outer dry and semi-dry layers) include the following: microwave-assisted extraction (MAE), pressurized hot water extraction (PHWE) or subcritical water extraction (SWE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), and ultrasound assisted extraction (UAE). MAE is a novel technique that is recently gaining attention in the extraction of flavonoids because of its applicability on biomaterials, rapid extraction time, higher quality of extract, and higher extraction recovery (Orsat & Routray, 2017; Stalikas, 2007). PHWE or SWE employs water at high temperatures (100–374 °C) and pressures such that the water remains in its liquid state as it extracts the solute (Castro-Puyana et al., 2013). As such, high pressure needs to be constantly maintained to ensure subcritical water conditions (Ameer et al., 2017). PLE is similar with PHWE except that it uses other solvents for extraction (Castro-Puyana et al., 2013). Though PLE gives high extraction yield and reduced solvent consumption, it is unsuitable for thermolabile compounds and the solvent needs to be carefully

chosen (Yahya et al., 2018). The SFE method, on the other hand, employs a fluid in its supercritical state, which behaves as a heavy liquid but with better transport properties and can penetrate like a gas, thus enhancing extraction of target compounds (Yahya et al., 2018). The solute that can be extracted by this method may be limited by the type of solvent used, such as carbon dioxide (CO₂), a nonpolar compound (Ameer et al., 2017). UAE uses ultrasound waves to facilitate disruption of the cell wall of the sample to liberate the target solute, resulting in reduced time, power and solvent consumption (Yahya et al., 2018). However, the high ultrasound waves may damage the active constituents in the sample and cause undesirable changes in extracted components (Ameer et al., 2017).

In the pursuit of finding alternative ways of utilizing onion wastes, particularly onion leaves, this study was done to investigate the extraction of bioactive compounds such as flavonoids and phenolics from waste onion leaves using aqueous ethanol as solvent via conventional solvent extraction (CSE) and microwave-assisted extraction (MAE). Ethanol is preferred over other solvents because of its efficiency, reliability, and application on food systems (Dorta et al., 2012; Lee et al., 2014; Safdar et al., 2017; V. Sharma & Janmeda, 2017; Yan et al., 2015). Parametric studies were performed for each method to study the effects of ethanol concentration (50 %v/v and 80 %v/v), temperature (60 °C and 80 °C), and extraction time (1 hr and 4 hrs), for CSE;



Figure 1. Dried onion leaves in the dehydrator (a) and dried onion leaves powder (b).

and those of ethanol concentration (50 %v/v and 80 %v/v), power level (10 % and 50 %), and extraction time (10 sec and 60 sec), for MAE, on the total flavonoid yield (TFY) and total phenolic yield (TPY) of the extracts from waste onion leaves. The methods were then compared in terms of the yield and recovery of the bioactive compounds.

MATERIALS AND METHODS

Experiments were conducted at the Department of Chemical Engineering, College of Engineering and Agro-Industrial Technology, University of the Philippines Los Baños. Fresh waste onion leaves from Red Creole variety were acquired from Occidental Mindoro, Philippines. The leaves were dried in a liquefied petroleum gas (LPG) dehydrator (no brand, Labotech Trading) at 55 °C for 12 hours or until the mass of the samples became constant. Then, the leaves were ground using a blender (600W Personal Blender, NutriBullet®, U.S.A.) before storing in a Ziploc® bag. Lastly, the ground samples were kept in a desiccator to avoid re-absorption of moisture. **Figure 1** shows the dried onion leaves in the dehydrator and the dried onion leaves powder with a particle size of less than 595 µm (undersize of mesh 30).

Conventional Solvent Extraction

For the conventional solvent extraction, one (1) g of dried onion leaves powder was mixed with 20 mL of the solvent in a 250-mL Erlenmeyer flask to keep a 1:20 sample-to-solvent ratio (Chan et al., 2011; Patil et al., n.d.). A reflux condenser was then attached to the Erlenmeyer flask, as shown in **Figure 2**, to avoid depletion of the solvent. Then, the mixture was stirred at a constant speed of 300 rpm as it was heated in a water bath at the assigned level of temperature, which was monitored every 30 min using a thermometer. Then, the effects of three parameters: ethanol concentration, temperature, and extraction time were tested using the two-level factorial experimental design. **Table 1** shows the summary of the high and low levels of each parameter: 50 %v/v and 80 %v/v for ethanol concentration; 60 °C and 80 °C for the temperature; and 1 hr and 4 hrs for the extraction time. These levels were chosen based from the optimized

conditions in literature (Jin et al., 2011; Yan et al., 2015). The runs were done in duplicate and 4 center points were added to check for curvature, generating a total of 20 runs.

After extraction, the mixture was immediately filtered using an ordinary filter paper into a clean 250-mL Erlenmeyer flask, to separate the extract from the filter cake. Then, the extract was diluted to mark, using the respective concentration of solvent (50 %v/v, 65 %v/v, and 80 %v/v), in a 25 mL volumetric flask. Lastly, the extract was transferred to a 50 mL Falcon® tube and was stored in a refrigerator at 4 °C until analysis.

Microwave-Assisted Extraction

Similar to the conventional method, one (1) g sample was mixed with 20 mL of aqueous ethanol solvent in a 250-mL beaker. The mixture was irradiated at specified power level and irradiation time. The power level was manipulated in the setting of the microwave used in the experiment. Similar to the conventional method, the mixture was immediately filtered using an ordinary filter paper, into a clean 250-mL Erlenmeyer flask, to separate the extract from the filter cake after the extraction. Then, the extract was diluted to mark, using the respective concentration of solvent (50 %v/v, 65 %v/v, and 80 %v/v), in a 25 mL volumetric flask. Lastly, the extract was transferred to a 50 mL Falcon® tube and was stored in a refrigerator at 4 °C until analysis.

The high and low levels of the three parameters observed for MAE were 50 %v/v and 80 %v/v for ethanol concentration, 10 % and 50 % for power level, and 10 sec and 60 sec for extraction time, as summarized in **Table 2**. Parameters were chosen based from the optimized conditions in literature and some preliminary testing. A two-level factorial design was also employed, with duplicate runs and 4 additional center points to check for curvature, for a total of 20 runs.

Total Flavonoid Yield (TFY) Determination

The aluminum chloride (AlCl_3) colorimetric method was used to get a quantitative measure of the total flavonoid yield (TFY) of the extracts (Chang et al.,

Table 1. Summary of the low and high levels of different factors for conventional solvent extraction.

FACTORS	LEVELS		SOURCE
	Low (-)	High (+)	
Ethanol Concentration (%v/v)	50	80	Chan et al. (2011)
Temperature (°C)	60	80	Chan et al. (2011); Yan et al. (2015)
Time (hrs)	1	4	Yan et al. (2015)

Table 2. Summary of the low and high levels of different factors for microwave-assisted extraction.

FACTORS	LEVELS	
	Low (-)	High (+)
Ethanol Concentration (%v/v)	50	80
Power Level (%)	10	50
Time (sec)	10	60

Reference: Chan et al., 2011

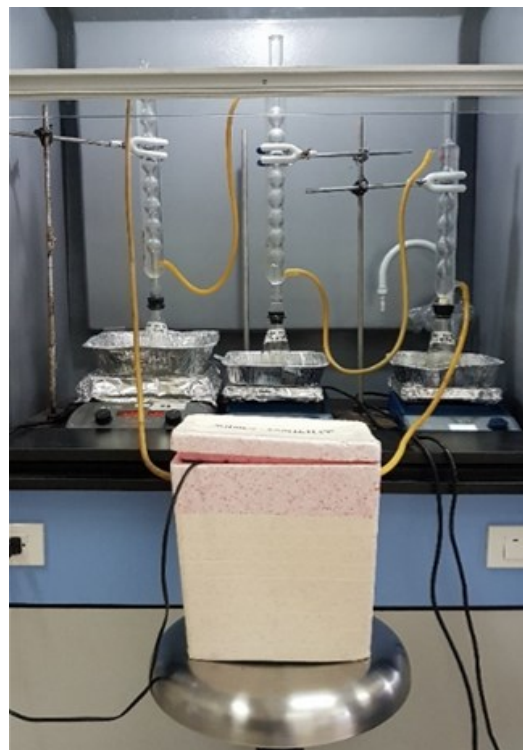


Figure 2. Conventional solvent extraction set-up.

2002). From the extract, 0.5 mL was transferred to a test tube using a pipettor. Then, 1.5 mL pure methanol, 0.1 mL 10 % AlCl_3 , 0.1 mL 1M potassium acetate (KCH_3COO), and 2.8 mL distilled water were added. A bright yellow solution was produced, which confirmed the presence of flavonoids. After allowing the solution to stand for 10 mins at room temperature, the absorbance was read at 430 nm using a spectrophotometer (UV-1280, Shimadzu, Japan).

Calibration curves were also obtained using quercetin as standard, which was prepared in six concentrations (0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL, 0.10 mg/mL, 0.12 mg/mL) which were also subjected to the same treatment as the sample. To compute for TFY, the absorbance of the sample was plotted in the calibration curve to obtain its concentration. Equation 1 was then used to calculate the TFY of the extracts, expressed as quercetin equivalent in mg/g dry weight (mg QE/g DW).

$$TFY = \frac{C_{QE} \times V}{m_{spl}} \quad \text{Equation 1}$$

where:

C_{QE} is the concentration from the calibration curve, mg/mL

V is the total volume of the extract, mL

m_{spl} is the mass of the sample (dry basis), g

Total Phenolic Yield (TPY) Determination

The TPY of the extracts was quantified using the Folin-Ciocalteu reagent (FCR) colorimetric method (Singleton et al., 1999). The reagents, 1.25 mL of 10 % FCR solution and 2.5 mL of 75 g/L NaCO_3 , were added to 0.25 mL of the extract, after which it was diluted to 25-mL with distilled water. A blue-colored solution was observed, which confirmed the presence of phenols. After shaking, the solution was allowed to stand in the dark for 2 hours at room

temperature before reading the absorbance at 760 nm in a spectrophotometer (UV-1280, Shimadzu, Japan).

Calibration curves were constructed using gallic acid as standard. Different concentrations (0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL, 0.10 mg/mL, 0.12 mg/mL, 0.14 mg/mL, 0.16 mg/mL, 0.18 mg/mL) of the standard solution were prepared, which underwent the same treatment as the sample.

The TPY of the sample was calculated by plotting the observed absorbance in the calibration curve. Equation 2 was then used to calculate the TPY of the extracts, expressed as gallic acid equivalent in mg/g dry weight (mg GAE/g DW).

$$TPY = \frac{C_{GAE} \times V}{m_{spl}} \quad \text{Equation 2}$$

where:

C_{GAE} is the concentration from the calibration curve, mg/mL

V is the total volume of the extract, mL

m_{spl} is the mass of the sample (dry basis), g

Determination of the Maximum Extractable Flavonoid and Phenolic Content

To determine the maximum extractable flavonoid and phenolic content from the samples, Soxhlet extraction was performed based from a standard procedure (Redfern et al., 2014), in which a round-bottom flask, Soxhlet apparatus, reflux condenser, rubber tubing, pump, ice bath, and hot plate were assembled. After setting up the needed equipment, one (1) g of dried onion leaves powder was securely wrapped in a filter paper, in replacement for a thimble, and then placed in the Soxhlet apparatus. After this, 150 mL of 95 % ethanol was poured into the round bottom flask and was heated to its boiling point on a hot plate. The highest concentration

available for the solvent was chosen for the Soxhlet extraction because it has the lowest boiling point, at around 78 °C, compared to its aqueous form. This was necessary to minimize the degradation of flavonoid and phenolic compounds at higher temperatures at the prolonged extraction time of Soxhlet extraction (Sagar et al., 2018). The procedure ran for approximately 18 hrs or until a clear color of the solvent was observed when contacted with the sample, which indicated that the extraction was already complete. The extract was then analyzed for its TFY and TPY and the percent recovery was calculated using the following equation:

$$\% \text{ recovery} = \frac{\text{TFY or TPY of sample}}{\text{maximum TPY or TFY}} \times 100 \quad \text{Equation 3}$$

Data Analysis

Design Expert® 11 (trial version) was used for the statistical analysis of this study. The effects of each parameter (ethanol concentration, temperature (for CSE)/ power level (for MAE), and extraction time) and their interactions on the responses (TFY and TPY) were observed; and the significant factors were determined using analysis of variance (ANOVA) at 95 % confidence level. Furthermore, abnormalities with the data were observed in the diagnostic tests which would determine the

compliance of the model to the four assumptions of ANOVA.

RESULTS AND DISCUSSION

Conventional Solvent Extraction: Effect of Parameters on TFY

During CSE, the highest TFY (2.63 mg QE/g DW) was obtained at high ethanol concentration (80 %v/v ethanol), high temperature (80 °C), and low extraction time (1 hr). ANOVA results showed that for CSE, the two factors (ethanol concentration and extraction time) as well as the two-way and three-way interactions of all the factors significantly affected the TFY of the extracts ($p \leq 0.05$). Flavonoids, which are mostly in their glycoside form (Shi et al., 2016), are more soluble in aqueous alcoholic solutions than in pure solvents (Orsat & Routray, 2017). These compounds cannot be completely extracted in the absolute concentration of organic solvents (ethanol in this case) as proteins and polyphenols in the plant matrix are linked by strong hydrogen bonds. These bonds, however, can be weakened by adding water to the solvent and therefore, accelerate desorption of the solute from the sample matrix (Mustafa & Turner, 2011). However, too much water in the solvent encourages the extraction of more polar compounds in the plant matrix, like mucilage, which interferes with the

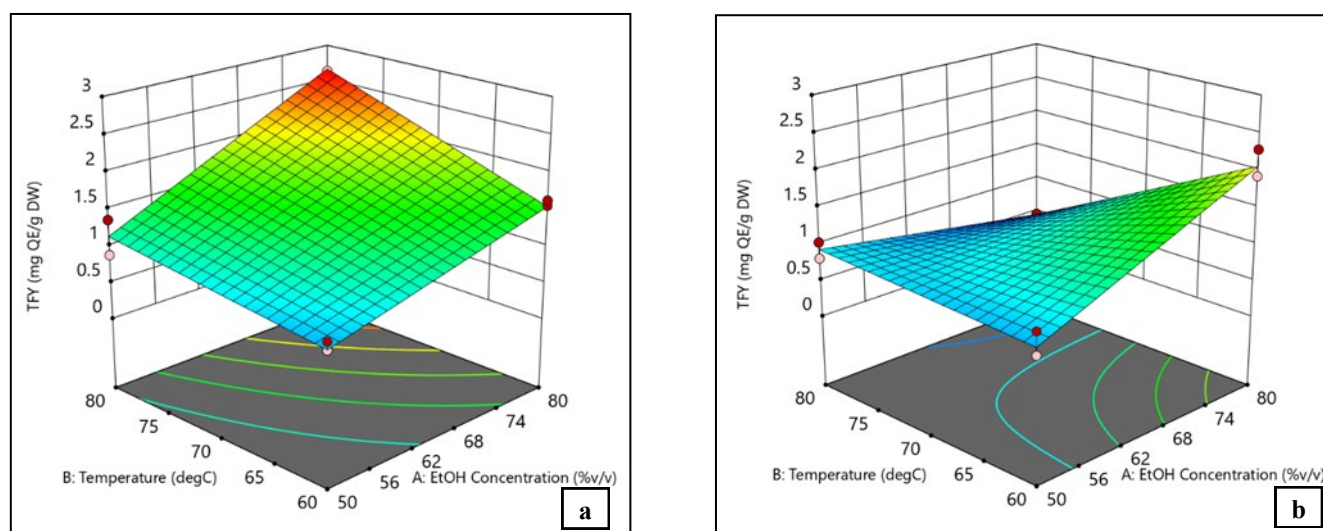


Figure 3. 3D surface plot for TFY by conventional solvent extraction at low (a) and high (b) extraction time.

diffusion of flavonoids to the solvent. Mucilage are highly hydrophilic substances that captures water and other molecules with their cave-like structures, forming gel-like substances (Bone & Mills, 2013). In the experiment, formation of these gel-like substances was observed as the extracts cooled down, particularly in the extracts with lower concentrations of ethanol. Thus, the higher concentration of ethanol (or lower water content) resulted in a higher extraction yield.

As shown in Figure 3a, increasing TFY was observed with increasing ethanol concentration and temperature at the low level of extraction time (1 hr). This could be attributed to the higher solubility of flavonoids in higher concentrations of ethanol and the increased diffusivity of solute at higher temperatures (Orsat & Routray, 2017). However, at longer extraction time of 4 hrs (Figure 3b), there was an observed decrease in TFY yield with increasing ethanol concentration at the higher level of temperature. Prolonged extraction time could have caused decomposition of flavonoids and degradation of compounds in the plant matrix from long exposures to such temperature level (Miean & Mohamed, 2001).

Conventional Solvent Extraction: Effect on TPY

For the phenolics, all the three factors, ethanol concentration-temperature interaction and ethanol concentration-extraction time interaction significantly affected the TPY ($p \leq 0.05$). For the phenolics, the highest TPY (6.15 mg GAE/g DW) was obtained at high ethanol concentration (80 %v/v ethanol), low temperature (60 °C), and low extraction time (1 hr).

As shown in Figure 4a, at low ethanol concentration (represented by the black line), increasing the temperature and extraction time had no effect on TPY, while at high ethanol concentration (shown in the red line), a decrease in TPY was observed with increasing temperature and extraction time. A higher temperature should increase the diffusivity of the solute. However, according to Miean and Mohamed (2001), some phenolic compounds are less thermally stable than flavonoids and start to decompose at around 60 °C, and they can also be easily oxidized or decomposed by light (Dmitrienko et al., 2012). Thus, the observed decrease in the phenolics yield.

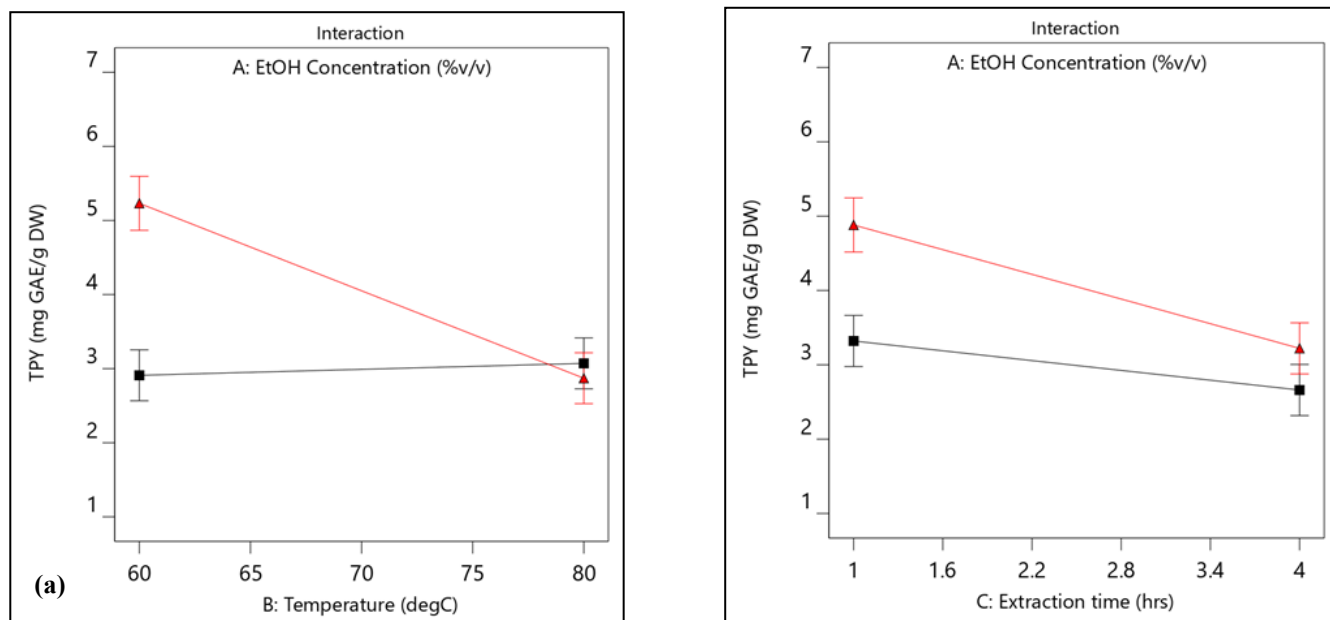


Figure 4. Effect of the interaction of ethanol concentration and temperature (a) and ethanol concentration and time (b) on TPY by conventional solvent extraction.

From Figure 4b, at lower ethanol concentration (shown in black line), increasing the extraction time had no effect on TPY, as indicated by the overlap in the error bars for the data points at 1 hr and 4 hrs. This could be due to the lower solubility of the solute in lower concentrations of ethanol; and so, the rate of diffusion may take longer (Alara et al., 2018). Due to this, increasing the contact time of the solvent and the sample did not have a significant effect on the amount of extracted phenolics. On the other hand, TPY decreased with extraction time at the higher ethanol concentration (see red line in Figure 4b). Longer extraction time could have caused the phenolic compounds to be oxidized or be decomposed by light (Dmitrienko et al., 2012). Higher extraction time also causes excessive heating of the samples which also denatures some of the phenolic compounds within the plant matrix (Dahmoune et al., 2014).

Microwave-Assisted Extraction: Effect on TFY

For MAE, the highest TFY (2.98 mg QE/g DW) was obtained at high ethanol concentration (80 %v/v

ethanol), high power level (50 %), and high level of extraction time (60 sec). The significant factors were ethanol concentration and power level.

Figure 5 shows the TFY during MAE at varying levels of ethanol concentration and power level. The TFY values increase with ethanol concentration, since flavonoids are more soluble in the organic solvent. However, a pure concentration of solvent is discouraged in MAE because its low polarity makes it a poor absorbent for microwave heating. For this reason, there are limited organic solvents that are effective in MAE, such as ethanol, methanol, and acetone. Generally, ethanol is said to be the most used solvent, because it can absorb microwave energy well and is also efficient in extracting many active compounds from plants (Chan et al., 2011).

In order to allow more absorption of microwave energy, water is usually added to the organic solvent. Water increases the heating efficiency of the mixture, and it enhances the solvent's penetration to the plant matrix (Dmitrienko et al., 2012). However, too much water is not

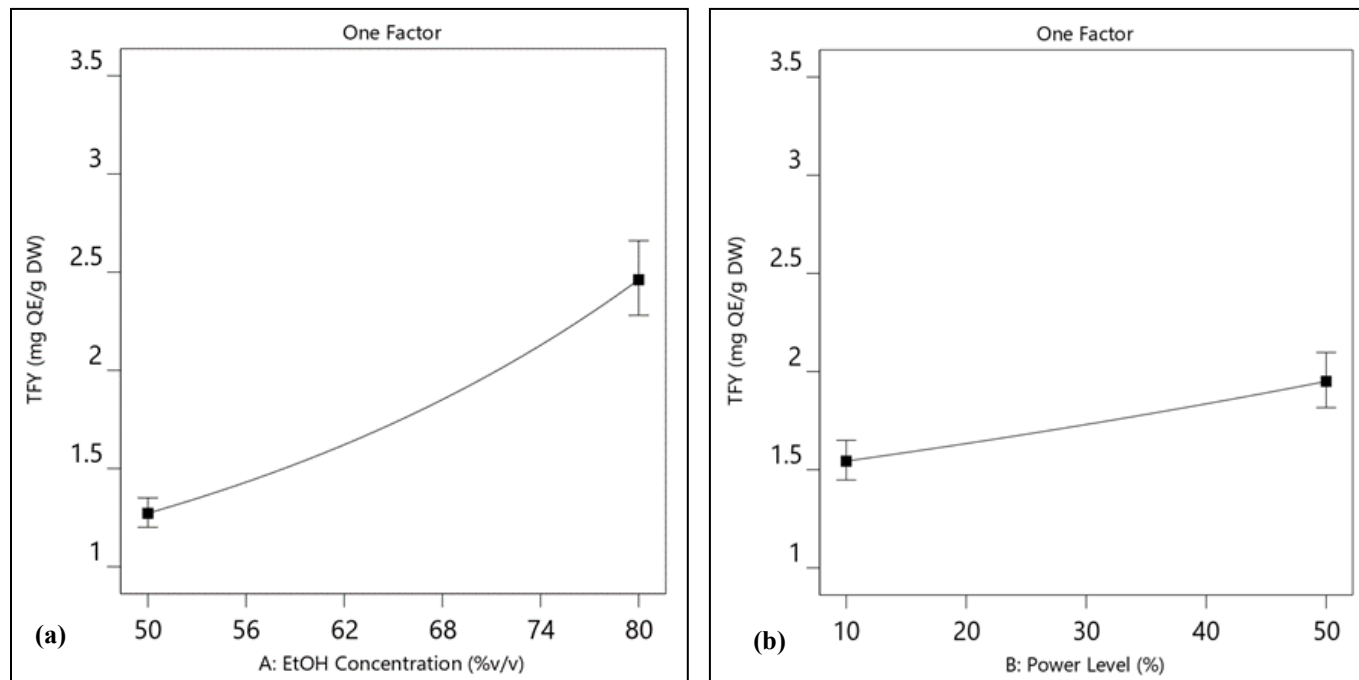


Figure 5. Effect of ethanol concentration (a) and power level (b) on the TFY by microwave-assisted extraction.

recommended to avoid the extraction of undesired polar compounds, such as mucilage, that could interfere with the extraction of the desired flavonoid compounds (Tosif, et al., 2021). This explains the decrease in the values of TFY at the lower concentration of ethanol, as presented in Figure 5a.

Power level also had a significant positive effect on TFY with a p-value equal to 0.0023. It had a positive effect on the response, as shown in Figure 5b, because the increase in power level promotes the disintegration of the cellular structures within the biocellular matrix, as both temperature and pressure increase in a short period of time due to dielectric heating (Routray & Orsat, 2014).

Power level is also directly related to temperature, since the latter is dependent on the combination of power level and time in the microwave setting. Higher power level may translate to higher extraction temperature, which then increases the solubility of the analyte in the solvent. Lower levels of microwave power can be compensated with a longer time setting, and vice versa, to achieve the same recovery of flavonoids (Mandal et al., 2007). This was observed in the experiment when the temperature of 50 %v/v ethanol increased from 37.0 °C to 40.9 °C as extraction time increased from 10 sec to 60 sec, at 10 % power level.

As mentioned earlier in CSE, a risk for thermal degradation of flavonoids and deterioration of the biological matrix may occur if the extraction temperature is beyond the optimum; and thus, it is the same for power level. Nevertheless, flavonoids are reportedly stable up to 110 °C (Chan et al., 2011). If an increase in TFY recovery is desired, the

power levels used in the experiment could still be increased, since the highest extraction temperature measured in MAE is 69.5 °C. In general, power level and time should be tuned to attain the right temperature that would produce the highest extraction of flavonoids (Orsat & Routray, 2017).

Microwave-Assisted Extraction: Effect on TPY

For MAE, the highest TPY (2.98 mg QE/g DW) was obtained at high ethanol concentration (80 %v/v ethanol), high power level (50 %), and high level of extraction time (60 sec). The significant factor was found to be ethanol concentration only.

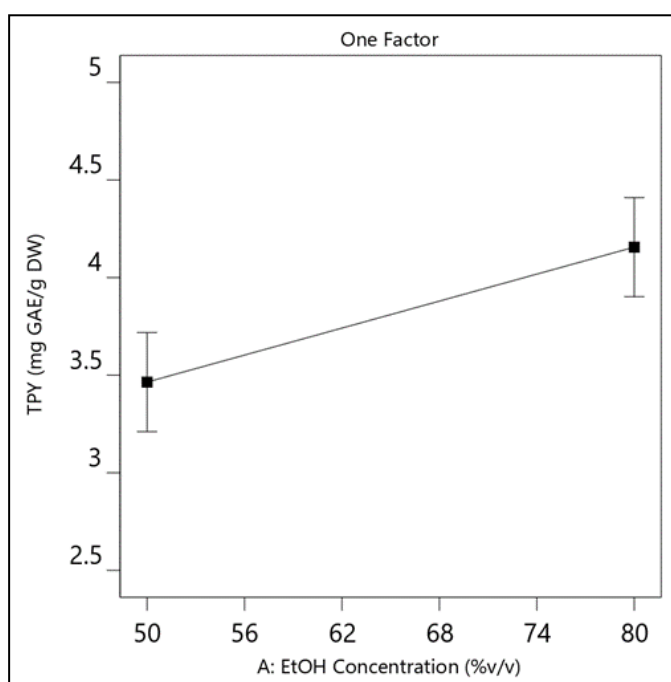


Figure 6. Effect of ethanol concentration on the TPY by microwave assisted extraction.

Table 3. Comparison of the highest average TFY and TPY recovery for conventional solvent extraction and microwave assisted extraction.

EXTRACTION METHOD	AVE. TFY (mg QE/g DW)	PARAMETERS	AVE. TPY (mg GAE/g DW)	PARAMETERS
CSE (This study)	2.63	80 %v/v aqueous ethanol, 80 °C, 1 hr extraction time	6.15	80 %v/v aqueous ethanol, 60 °C, 1 hr extraction time
MAE (This study)	2.98	80 %v/v aqueous ethanol, 50 % power level, 60 sec extraction time	4.24	80 %v/v aqueous ethanol, 50 % power level, 60 sec extraction time

As shown in Figure 6, ethanol concentration had a positive effect on the response, which was consistent with the previous results, and with reasons already mentioned in the earlier discussions. The type of solvent used and the amount of water present in the solvent are the main parameters that affect MAE; because extraction recovery greatly depends on the solubility of the target compounds to the solvent, and the efficiency of the extraction is dependent on the solvent's ability to absorb microwave energy, which is aided by the presence of water in the solvent (Zhang et al., 2011).

Comparison of Conventional Solvent Extraction and Microwave Assisted Extraction

Table 3 shows the highest TFY and TPY for the two methods, which were obtained at the specified conditions. In comparison with the results from Soxhlet extraction, the obtained results corresponded to around 30.6 % and 34.7 % flavonoids recovery, for CSE and MAE respectively, and to 68.7 % and 47.3 % for phenolics recovery, for CSE and MAE respectively. The results are also comparable to those reported in literature by Nile et al. (2018) for onion solid waste extraction using the same solvent (TFY = 4.82 mg QE/g dw; TPY = 6.12 mg GAE/g dw). Based on a studentized two-tailed t-test, all p-values were greater than 0.05, indicating that there were no significant differences among the TFY and TPY values from the two methods in this study and those obtained by Nile et al. (2018).

The significance of the difference between the means of both TFY and TPY for CSE and MAE was determined through a studentized two-tailed t-test. For TFY, results of the analysis indicated a t-value of -1.586 and a p-value of 0.1349, indicating that there was no significant difference between the means of TFY obtained from CSE and MAE at 95 % confidence level. In terms of TPY, the results of the studentized two-tailed t-test, showed that, with a t-value of -0.5232. and a p-value of 0.6090, the difference between the TPY means of CSE and MAE was also not significant. This indicates that MAE can extract as much flavonoids and phenolics

from waste onion leaves as with CSE, but at a very much shorter extraction time (MAE: 60 sec < CSE: 1 hr). A shorter extraction time could also lower the overall extraction cost because of less power consumption.

Some studies have reported greater extraction of flavonoids and phenolics using MAE than the conventional methods (Dorta et al., 2013; Jin et al., 2011). The major difference between these methods is the exposure of the extracts at high temperatures for the conventional method. To boost the extraction recovery, it is recommended to operate at a higher temperature because it increases the diffusivity of the analytes to the solvent. But exposing the samples in high temperature for a long period of time would lead to the degradation of the desired compounds and disintegration of the biological matrix. Thus, using MAE is advantageous for this reason, because it can raise the temperature of the sample at a very short period of time (Jin et al., 2011).

Another advantage of MAE is the presence of microwave energy that provides localized heating and pressure build up, which leads to cell disruption. Microwave does not only heat the solvent, but also targets the moisture content of the plant sample. As the energy of the water inside the plant increases abruptly, the cellular matrix disintegrates and the desired compounds leach out to the solvent (Chan et al., 2011; Orsat & Routray, 2017). The power level of microwave energy can be further explored and maximized so that other parameters, such as extraction time and volume of solvent, can be decreased to lessen the overall extraction cost.

CONCLUSION

This study showed that extraction of flavonoid and phenolic compounds from waste onion leaves via CSE depends on ethanol concentration, extraction time, and the two-way and three-way interaction of all the factors for the flavonoids; and all the three factors, ethanol concentration-temperature interaction and ethanol concentration-extraction time interaction for the phenolics. On the other hand, for MAE, the extraction yield was

significantly affected by the ethanol concentration and power level only for the flavonoids and the ethanol concentration only for the phenolics.

Increasing the extraction temperature is a determining factor to obtain high extraction yields, but over-exposure to such condition could lead to damage to the bio-cellular matrix and denaturation of heat-sensitive compounds in the leaves. As such, MAE was found to be a more advantageous extraction method than conventional method, because of its comparable yield and recovery at a shorter extraction time. Overall, the waste onion leaves could be utilized as a source of phenolics and flavonoids, transforming them into potential feedstock in the production of high value products such as these bioactive compounds.

RECOMMENDATIONS

For future studies, a higher range of power level and time could be applied in MAE to determine if the yields could be increased further. Also, solvent recycling and/or lower solvent loading could be explored to lessen the overall cost. As a next step, optimization studies could also be done to maximize the yields of phenolics and flavonoids.

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