Chapter-3

Effect of microwave and enzymatic pretreatment and type of solvent on kinetics of ultrasound assisted extraction of bioactive compounds from ripe papaya peel

Chapter 3

Effect of microwave and enzymatic pretreatment and type of solvent on kinetics of ultrasound assisted extraction of bioactive compounds from ripe papaya peel

---

3.1 Introduction

Tropical fruits are becoming more popular in both local and global markets due to their nutritional and medicinal properties. When tropical fruits are processed to extract desirable components from other plant tissues, leftover materials such as peel, seeds, and inedible pulp are generated as byproducts **[4]**. The disposal of these byproducts is a critical issue in regards with waste management, environmental concerns, legal limits, and financial constraints **[37]**. Using such wastes in the creation and development of functional meals or nutraceutical components is currently one option for reducing this problem. Byproducts are a source of naturally occurring components such as, phenolic compounds, flavonoids, vitamin C, and carotenoids, which have antioxidant properties and are linked with health benefits **[42]**.

Papaya (*Carica papaya* Linn) is a type of tropical fruit that is categorized under the *Caricaceae* family and due to its medicinal characteristics; it has been utilized as therapeutic remedy. In English, it's called papaya, in Hindi, Papita, and in Sanskrit, Erandakarkati. The plant originates from North America and was brought to India in the 16th century **[48]**. Papaya is a widely consumed fruit because of its delicious taste and great nutritional content. It contains lots of vitamins, notably vitamin C **[6]**. Because of its unique enzymes papain and bioactive substances, the papaya plant has to find usage as a valuable medicinal plant in addition to its nutritional characteristics.

Papaya has a large export market due to its excellent nutritional value in the tropical fruit sector globally. After mango and pineapple, papaya is presently placed third with 15.36% of total tropical fruit output. Papaya has a global production of 13.4 million metric tons. India's production capacity is 6.39 million metric tons. On the basis of state-by-state value of papaya production, Andhra Pradesh is the first major producer and Gujarat is the second largest **[15]**.

In the food processing sector, papaya is also frequently employed as a valued raw material. During processing, 8.47% of skin, 6.51% of seeds, 32% of unsuitable pulp, and 52.96% of the finished product are obtained **[4]**. The production of such waste material indicates that obtaining additional value for all of these byproducts would be extremely beneficial to papaya producers. Characterization of different segments of papaya, such as their peels and seeds are an excellent approach to show that their total consumption may provide a significant quantity of essential elements like vitamins and bioactive substances **[18]**. Phenolic acids, carotenoids, and flavonoids are among the primary bioactive components found in papaya pulp, peel, and seeds. The bioactive components found in edible papaya and its derivatives make them appeal culinary components, particularly for the development of functional foods and nutraceutical ingredients.

Furthermore, papaya peel contains fiber, phenolic compounds, soluble solids, vitamin C, and the minerals potassium, copper, and salt **[25]**. Papaya peel is typically considered a trash and might represent an environmental hazard in overproduction. It is frequently abandoned since it receives little consideration in terms of utilization or recycling. Fruit by-products such as peel and seed segments, for example, may contain numerous useful chemicals such as phenolics, flavonoids, and carotenoids, and, like the fruit pulp itself, may have a high antioxidant potential **[21]**. While the fruit pulp of the papaya has nature's most dense source of carotenoids, particularly lycopene, which has great antioxidant properties **[30]**, the fruit peel also possesses high antioxidant properties and phenolic content **[19]**. The antidandruff, skin healing, and moisturizing properties of papaya peel is an essential commodity in the cosmetic industry.

In recent years, there have been a large number of papers based on the separation of phyto-constitutes from natural sources, including total phenolic compounds. However, other from a few publications on detecting total phenolic contents in papaya peel, little work on extracting phenolic compounds from papaya peel has been done to date. There is also only little data available on the kinetics of phenolic components extraction from papaya peel. As the extraction efficacy and extract quality is influenced by a variety of process factors, a systematic study and engineering strategy for extraction, particularly of peel, was required. The kinetic behavior of solute extraction from bioactive compounds, which involves solute release through permeable channels and transport to the solvent stage, is also governed by the mass transfer phenomena **[15]**. Furthermore, the kinetic

analysis aids in the reduction of energy usage and the comprehensive design of the process for industrial scale-up **[36]**.

Therefore, the extraction conditions must be carefully considered in order to isolate these important natural compounds in a usable and intact state. The extraction process is critical for getting an intact and high yield of a certain components from plant materials in order to demonstrate their biological activity. As a result, it is critical to figure out the effective processing conditions such that the maximum amount of these components can be extracted without degradation and in a cost-effective way so that they may be used in other applications. This form of bio-prospecting examination on crude extracts before isolation of pure compounds from crude blends can assist to offer a prior indication on what kind of qualities it contains that may be employed to decord problematic situations.

Firstly, in this study, the proximate composition and mineral element profile of papaya peels were explored. The main purpose of this study was to see how different parameters (pretreatment, solvent, and extraction time) and ultrasonic extraction affect bioactive compounds and antioxidant capacity in papaya peel, and to determine the best extraction method. The purpose was to look at the kinetics of the extraction process and to assess the applicability of the most often reported kinetic models in the literature.

3.2 Material and Methods

3.2.1 Sample collection

Papaya fruits (variety “Khasi Dwarf Papaya”) were bought from the Tezpur Fresh Fruit and Vegetable Market and brought to the Tezpur University of Assam's Food Engineering and Technology Department. They were chosen based on their ripening stage (the point at which the fruit is ready to eat), color consistency, average size, and lack of flaws. Clean water was used to wash the fruits that were chosen. They were then manually peeled, the skins were peeled off, the seeds were removed, and the mucilage was rinsed away with running tap water.

3.2.2 Chemical and reagents

Viscozyme, Folin-Ciocalteu reagent 2N (Sigma-Aldrich), DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Sigma-Aldrich), gallic acid (Sigma-Aldrich), sodium carbonate

anhydrous, ferric ammonium sulfate, sodium bicarbonate (Himedia, India), methanol and ethanol were obtained from Zenith India, Assam.

3.2.3 Proximate and mineral analysis

The proximate analysis of papaya peel powder involved the assessment of various nutritional components using standard AOAC methods **[3]**. Fat content was determined through solvent extraction to isolate and quantify the fat present in the papaya peel powder.

The ash content was assessed by incinerating the powder to eliminate organic matter, leaving behind the inorganic mineral content, which is commonly referred to as ash.

To determine the crude protein content, the Kjeldahl method for protein analysis was employed, involving sample digestion and subsequent quantification of nitrogen content, with protein content calculated based on the nitrogen content.

The carbohydrate content was estimated by subtracting the cumulative percentages of fat, ash, crude protein, and crude fiber from 100%, providing an approximation of the carbohydrate content within the papaya peel powder.

Mineral analysis was performed using Atomic Absorption Spectrometer (ICE3000, Thermo Fischer scientific, USA).

3.2.4 Extraction Process

A combined extraction technique made up of different pretreatments (such as microwave, enzyme, microwave-enzyme, and enzyme-microwave) and an ultrasound bath was used to carry out the extractions. As per preliminary trials, 0-2 mL of viscozyme was used for enzyme pretreatment (1 h) and found that concentration of the enzyme below 1 mL gave unsatisfactory results or poor extraction and concentration of viscozyme above 1 mL gave unnoticeable changes in the extraction when compared with 1 mL concentration. Only 1 mL concentration of viscozyme gave satisfactory results for extraction. For microwave pretreatment, 900 W was taken as constant parameter and time of the pretreatment was varied. But as the time exceeded 1 min, the solvent started

to boil and create problem in the extraction. So, 1 mL of viscozyme (Sigma-Aldrich) was used for enzyme pretreatment and microwave was done at 900 W for 1 min. Two different solvents and their mixtures were used after standardization to identify the most suitable one for the recovery of polyphenols from papaya peel. The solvents used (2:1) included water, ethanol, and their mixture (1:1). Ethanol and water were preferred over acetone and other solvents due to their safety, food-grade suitability, and their demonstrated effectiveness in extracting specific bioactive compounds found in papaya peel.

Table 3.1: An overview of the experimental conditions for several methods of extraction

Exp. No.	Combination	Pretreatment	Solvent
1	M-W	Microwave	Water
2	M-Et	Microwave	Ethanol
3	M-WEt	Microwave	Water: Ethanol (1:1)
4	E-W	Enzyme	Water
5	E-Et	Enzyme	Ethanol
6	E-WEt	Enzyme	Water: Ethanol (1:1)
7	ME-W	Microwave-Enzyme	Water
8	ME-Et	Microwave-Enzyme	Ethanol
9	ME-WEt	Microwave-Enzyme	Water: Ethanol (1:1)
10	EM-W	Enzyme-Microwave	Water
11	EM-Et	Enzyme-Microwave	Ethanol
12	EM-WEt	Enzyme-Microwave	Water: Ethanol (1:1)

M is microwave, E is enzyme, W is water, & Et is ethanol

The extraction from papaya peels was studied with and without use of ultrasound. In the case of ultrasonic assistance, extraction was done at constant temperature at 40°C for 0, 15, 30, 45, 60, 75 and 90 min and the sonication applied in continuous mode in ultrasonic bath (Genaxy Scientific – SK3300LHC) at a frequency of 53 kHz and a power

of 100 W. All extractions were done in triplicate. The following equation was utilized to determine the impact of ultrasound:

$$Effect\ of\ ultrasound\ (\%) = \frac{B - A}{B} * 100 \quad (1)$$

where, A represents yield of polyphenols extracted without ultrasonic energy (%) and B represents yield of polyphenols extracted with ultrasonic energy (%).

After ultrasound treatment, the extract is initially in liquid form, containing the dissolved bioactive compounds. This liquid extract serves as a practical medium for various analytical purposes, allowing the quantification of bioactive compounds and assessment of antioxidant activity. However, for long-term storage and application, the liquid extract undergoes further processing to obtain a dry extract. The preferred method for producing a dry extract is typically freeze-drying.

3.2.5 Quantitative Analysis

3.2.5.1 Total phenolic content

The Folin-Ciocalteu standard method, as described by Singleton & Rossi **[33]**, was used to determine the total phenolic content in papaya peel extracts, with slight modifications. A 0.5 mL sample of papaya peel extract (0.1g dissolved in 100 mL water) was diluted in 2.5 mL FC reagent in the test tube (diluted 1:10 with water). After 5 minutes, the test tube was filled with 2 mL of a 7.5% Na_2CO_3 solution. The tubes were incubated at room temperature (23±2°C) for 2 hours, and the absorbance was assessed using a UV-Vis spectrophotometer at 765nm against a blank reagent (Agilent Technologies, USA, Cary 60 UV Vis). The total phenolic content was measured in mg GAE per 100 g of dry weight.

3.2.5.2 Total flavonoid content

Aluminum chloride protocol was used to determine flavonoid content as per the methodology used by Saikia et al. **[35]**. Initially, a mixture was prepared by combining 0.5 mL of the peel extract with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum trichloride, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water. The resulting mixture was incubated for 40 minutes at room temperature (23±2°C). The absorbance of the reaction mixture was measured at 430 nm against a blank using a UV-Vis spectrophotometer (Agilent Technologies, USA, Cary 60 UV Vis). On a dry basis, the findings of the total flavonoid content were reported as mg QE/100 g.

3.2.5.3 Antioxidant activity (DPPH Assay)

The antioxidant activity of papaya peel extracts was assessed using the standard DPPH technique se per the methodology used by Kulkarni **[22]**. A mixture of 0.1 mL papaya peel extract and 3.9 mL DPPH solution was vigorously shaken for 2-3 minutes. After that, the mixture was incubated for 1 hour at room temperature (23±2°C) in the dark. The entire experiment was performed in complete darkness. Using a UV-Vis spectrophotometer, the extract's absorbance was measured at 517 nm (Agilent Technologies, USA, Cary 60 UV Vis). Methanol was used as a blank. Using the formula, the antioxidant scavenging capacity of papaya peel extract was determined.

$$Antioxidant\ Activity\ (\%) = \left(1 - \frac{A_{Sample}}{A_{Control}}\right) \times 100 \qquad (2)$$

where, A_{Sample} represents absorbance of sample, $A_{Control}$ represents absorbance of control

3.2.6 Kinetic modeling

The mass transfer process during solute extraction from a solid matrix may be explained using a several models, all of which are based on mass transfer integration.

During the extraction process, the solvent flow rate and physical characteristics remain unchanged. The following models are used to explain the mass transfer process during extraction.

3.2.6.1 Second order reaction

Because the solid–liquid extraction process is the polar opposite of adsorption, the concepts of adsorption kinetic equations may be used, and for the extraction rate, the second-order law proved to be the best fit **[30]**. According to Pan et al. **[29]**, the general second-order model is:

$$\frac{dC_t}{dt} = k * (C_e - C_t)^2 \qquad (3)$$

Where C_e refers to the equilibrium concentration of phenolic compounds in the liquid extract (g/L), Ct refers to the concentration of phenolic compounds in the liquid extract

at a certain extraction time (t), and k represents the rate constant of the second-order extraction rate constant (L/g min).

With boundary constraints t = 0 to t and Ct = 0 to Ct, Eq. (4) or a linearized Eq. (5) may be used to define the integral rate law for a second-order extraction **[29]**.

$$C_t = \frac{k * t * C_e^2}{1 + k * t * C_e} \quad (4)$$

$$\frac{t}{C_t} = \frac{1}{k * C_e^2} + \frac{1}{C_e} + \frac{1}{h} + \frac{t}{C_e} \quad (5)$$

where t approaches 0, h represents the initial extraction rate (g/L min):

$$h = k * C_e^2 \quad (6)$$

3.2.6.2 Langmuir model

Many researchers have frequently utilized two basic models, the Exponential and Langmuir models, to analyze the mass transfer process **[27]**. One of the most well-known models for explaining extraction kinetics is the Langmuir model. Although the adsorption model is most commonly used to investigate the extraction of polyphenol chemicals, it may also be used to examine the extraction of papaya peel matrix. After soaking the material in various solvents in an extraction vessel for a period of time, the solute moves out from the internal matrix and binds to the surface. Subsequently, the solute then moves to the separator vessel in the solvent.

$$Y = \frac{Y_f * t}{K_L + t} \quad (7)$$

Where, Y_f and K_L are constants (Y_f represents yield at infinite time), while Y represents % extraction yield (w/w).

3.2.7 HPLC analysis

For the confirmation, high-performance liquid chromatograpgy (HPLC) analysis was performed of extracted phenolic compounds from the best selected extraction technique with Waters HPLC system, USA with a Model: Waters 1525, 2414, 2489 operational system. The detection of phenolic compounds was conducted at a wavelength of 254 nm, utilizing a flow rate of 1 mL/min, while maintaining the column

temperature at 30°C. The separation process was carried out employing a dual pumping system, with varying ratios of 1% (v/v) acetic acid in milli-Q water (mobile phase A) and pure methanol (mobile phase B), both of high-performance liquid chromatography (HPLC) grade. A sample volume of 20 µL was injected, and the identification of phenolic compounds was achieved by comparing their retention time and spectral characteristics with those of established reference standards.

3.2.8 Statistical analysis

The obtained results of the samples (in triplicates) were analyzed through analysis of variance (ANOVA) test using IBM SPSS 24.0 software followed by Duncan test considering a 5% significance level (p-value ≤ 0.05).

3.3 Results and Discussion

3.3.1 Proximate and mineral composition

In order to fully utilize papaya peel, comprehensive knowledge of its physical and chemical properties is crucial. This information can facilitate the development of environmentally friendly approaches for papaya peel utilization. The proximate composition analysis revealed the following results for papaya peel powder (Table 3.2): ash content 5.98%, crude fiber 31.51%, crude protein 19.67%, fat content 2.51%, and carbohydrate 40.33%. The fat, protein, and ash contents align closely with the findings reported by Dotto & Abihudi **[13]**. The findings highlight the significant carbohydrate content present in papaya peels, indicating their potential as a valuable source of carbohydrates.

Table 3.2: Proximate chemical composition of papaya peels

SI. No.	Nutrient	Percentage (%)
1.	Protein	19.67±0.04
2.	Crude Fiber	31.51±0.03
3.	Carbohydrate	40.33±0.08
4.	Ash	5.98±0.03
5.	Fat	2.51±0.13

The mineral analysis revealed that papaya peels are notably abundant in potassium and calcium (Table 3.3). Potassium was found to be the most abundant mineral in the peels, with a concentration of 70.100 ± 0.82 mg/L, followed by calcium at 22.15 ± 0.13 mg/L. The levels of calcium, an essential element in chlorophyll, and magnesium, which serves as the central atom in the chlorophyll molecule, decrease as the papaya ripens **[31]**. Papaya peel also contains various trace minerals, including aluminum, chromium, copper, iron, magnesium, sodium, lead, and zinc, although their specific functions in the human body are not well-established. Due to its substantial mineral nutrient content, papaya peel can be regarded as a valuable source of minerals and may be utilized as an alternative food option.

As a result, regular consumption of papaya peel may not be advisable; however, extracts obtained from the peel, after appropriate processing, could serve as a viable source of minerals.

Table 3.3: Mineral composition of papaya peels

Minerals	Amount (mg/L)
Al	2.423
Ca	22.150
Cu	0.983
K	70.100
Mg	10.855
Na	2.179
Pb	0.090
Zn	4.236
Fe	0.146
Cr	0.063

3.3.2 Extraction yield

The effect of various pretreatments and solvents (Table 3.1) on ultrasonic extraction of polyphenol compounds from papaya peel and their extraction kinetics was investigated in this study. Ultrasonication is an effective way to boost with extraction since it dissipates enough energy for effective mass transfer. The ultrasonic energy is

considered to speed up the diffusion process by increasing the permeability of solid particles to the solvent, allowing for easier polyphenol release **[44]**. To conduct kinetic analysis, the data was fitted to a second-order extraction model and Langmuir model, and regression coefficients were calculated. The extraction capacity, C_e, the extraction rate constant, k, and the coefficient of determination, R^2, for the different pretreatments and solvents used were presented in Table 3.4. The release kinetics of total phenolics from papaya peel under ultrasonic action into a bath system were also described using the second-order kinetic model **[41]**.

The aim of the kinetic experiment was to do a critical assessment of the extraction characteristics between pretreatments and extraction solvents. Tables 3.4, Table 3.5 and Table 3.6 show the kinetic parameters that were derived analytically. In addition, the diffusivities of polyphenol compounds during extraction from papaya peel were determined. TPC, TFC and antioxidant activity of papaya peel extract were measured using adsorption kinetics in the first experiment, as well as to choose the pretreatment and solvent to be used during the next steps of the experiment. Extraction of total phenolic content, according to analysis of variance (Table 3.4), was significantly affected ($p \leq 0.05$) by pretreatment and solvent type, which demonstrates significance for the regression model. Based on the results of Table 3.4, the second-order model has a very high correlation coefficient R^2; and all are greater than 0.8119. The maximum coefficient of determination, R^2 in total phenolic content, was 0.9996 in E-W pretreatment indicating that 99.96% of the total variability in the response could be explained by both of the models used and shown that there was a strong correlation between the experimental and predicted values of yield followed by M-W pretreatment showed 0.9977 R^2 value. Thus, in this study, when changing pretreatment and solvent type, the second-order model predicted changes in TPC content during the extraction of papaya peel. Similarly, the regression equation obtained for total flavonoid content indicated the R^2 value of 0.9971 (99.71%) and for antioxidant activity, the R^2 value was 0.9998 (99.98%) in E-W treatment, which means enzyme-water pretreatment revealed that the model fits accurately the experimental data, which demonstrate significance for the regression model. According to the ANOVA analysis, pretreatment and extraction solvent had a substantial influence on extraction yield ($p \leq 0.05$). As shown by the high value of the coefficient of determination, the second-order model fit the experimental data well. This confirmed that there were two main stages during phenolics extraction

from papaya peel extract: The first step involves the fast dissolving of soluble constituents at particle surfaces into the solvent, followed by the gradual mass transfered of soluble components from the interior material into the solvent via the mechanism of diffusion **[9,43]**.

Table 3.4: Parameters of the Second order (k) and Langmuir (K_L) models for total phenolic content

Treatment	2nd order reaction rate constant (k)	Langmuir model rate constant (K_L)	C_e	R^2	RMSE
E-WEt	0.0025 ± 0.00036^{ef}	4.944 ± 0.698^{e}	80.58	0.9859	3.260
E-Et	0.0041 ± 0.00053^{cd}	2.460 ± 0.309^{f}	84.57	0.9974	1.774
E-W	0.0134 ± 0.00334^{a}	0.914 ± 0.215^{g}	98.90	0.9996	0.632
M-WEt	0.0035 ± 0.00009^{de}	4.009 ± 0.204^{e}	69.54	0.9828	3.120
M-Et	0.0058 ± 0.00204^{c}	2.538 ± 0.698^{f}	71.32	0.9965	1.501
M-W	0.0050 ± 0.00044^{cd}	2.308 ± 0.198^{f}	85.69	0.9977	1.458
ME-WEt	0.0014 ± 0.00016^{ef}	16.390 ± 1.323^{b}	42.56	0.9351	3.523
ME-Et	0.0016 ± 0.00007^{ef}	6.474 ± 0.242^{d}	46.37	0.9663	5.715
ME-W	0.0100 ± 0.00333^{b}	2.289 ± 0.708^{f}	93.98	0.9955	1.015
EM-WEt	0.0005 ± 0.00002^{f}	31.696 ± 0.929^{a}	58.73	0.8119	8.213
EM-Et	0.0018 ± 0.00017^{ef}	7.943 ± 0.663^{c}	68.93	0.9793	3.268
EM-W	0.0016 ± 0.00006^{ef}	6.857 ± 0.252^{d}	86.49	0.9872	3.254

values are expressed as mean ± standard deviations. Means in a same column with different superscripts indicate significant difference ($p < 0.05$).

Table 3.5: Parameters of the Second order (k) and Langmuir (K_L) models for total flavonoid content

Treatment	2nd order reaction rate constant (k)	Langmuir model rate constant (K_L)	C_e	R^2	RMSE
E-WEt	0.0030 ± 0.0004^{ef}	7.893 ± 1.116^{de}	42.34	0.9771	2.161
E-Et	0.0043 ± 0.0009^{de}	4.238 ± 0.584^{fg}	48.45	0.9859	2.189
E-W	0.0080 ± 0.0007^{a}	2.575 ± 0.196^{g}	55.53	0.9971	0.931
M-WEt	0.0026 ± 0.0003^{fg}	10.530 ± 1.660^{d}	36.19	0.9375	2.861
M-Et	0.0035 ± 0.0005^{def}	7.294 ± 0.903^{de}	38.98	0.9758	1.960
M-W	0.0062 ± 0.0014^{bc}	3.336 ± 0.607^{g}	48.91	0.9969	0.958
ME-WEt	0.0016 ± 0.0002^{g}	26.033 ± 2.314^{a}	22.94	0.8867	2.513
ME-Et	0.0069 ± 0.0018^{ab}	6.322 ± 1.554^{ef}	23.75	0.9693	1.320
ME-W	0.0049 ± 0.0009^{cd}	4.137 ± 0.643^{fg}	50.04	0.9948	1.256
EM-WEt	0.0021 ± 0.0004^{fg}	17.33 ± 3.000^{b}	27.64	0.9093	2.903
EM-Et	0.0016 ± 0.0000^{g}	13.45 ± 0.415^{c}	33.40	0.9624	2.804
EM-W	0.0032 ± 0.0005^{ef}	9.307 ± 1.218^{d}	44.55	0.9778	1.691

values are expressed as mean ± standard deviations. Means in a same column with different superscripts indicate significant difference ($p < 0.05$).

Table 3.6: Parameters of the Second order (k) and Langmuir (KL) models for antioxidant activity

Treatment	2nd order reaction rate constant (k)	Langmuir model rate constant (K_L)	C_e	R^2	RMSE
E-WEt	0.0078 ± 0.0015^{d}	1.425 ± 0.298^{de}	92.33	0.9989	1.066
E-Et	0.0083 ± 0.0007^{d}	1.356 ± 0.184^{de}	89.91	0.9981	1.361
E-W	0.0181 ± 0.0012^{c}	0.583 ± 0.041^{e}	94.79	0.9993	0.886
M-WEt	0.0112 ± 0.0002^{cd}	1.063 ± 0.188d^{e}	83.42	0.9991	0.909
M-Et	0.0090 ± 0.0006^{d}	1.301 ± 0.078^{de}	85.21	0.9993	0.762
M-W	0.0313 ± 0.0035^{a}	0.352 ± 0.069^{e}	92.68	0.9998	0.496
ME-WEt	0.0005 ± 0.00003^{e}	41.253 ± 2.101^{b}	43.29	0.7971	6.312
ME-Et	0.0008 ± 0.0001^{e}	23.796 ± 5.270^{c}	53.27	0.8497	7.100
ME-W	0.0228 ± 0.0031^{b}	0.479 ± 0.102^{e}	94.37	0.9997	0.546
EM-WEt	0.0004 ± 0.00003^{e}	60.663 ± 9.517^{a}	37.08	0.6341	8.007
EM-Et	0.0023 ± 0.00004^{e}	7.263 ± 0.397^{d}	58.33	0.9714	3.323
EM-W	0.0185 ± 0.0023^{bc}	0.588 ± 0.078^{e}	92.56	0.9998	0.461

values are expressed as mean ± standard deviations. Means in a same column with different superscripts indicate significant difference ($p < 0.05$).

The adsorption kinetics of polyphenols from papaya peel extract was described using the Langmuir kinetic model. As illustrated in Figure 3.1a, 3.1b and 3.1c, the adsorption kinetics was much faster for E-W than other pretreatments in total phenolic content and similar pattern was also observed for total flavonoid content (Figure 3.2). With correlation values of 0.9996 in TPC, the Langmuir model also correlates well with experimental data (Table 3.4). During 90 minutes of contact time, an asymptotic curve was obtained, and an adsorption/desorption equilibrium was formed for E-W, but after the same contact time for other pretreatments, only about half of the available polyphenols had been adsorbed. As a result, in this investigation, a contact period of 90 minutes was chosen appropriate since it enabled viewing the variations in adsorption strength between the different pretreatments and solvent interaction. It was discovered that when the amount of ultrasonic energy is increased, the rate of extraction rises.

From Table 3.4, Table 3.5 and Table 3.6 the values of TPC, TFC and antioxidant activity were plotted as kinetic curve with extraction time for the different pretreatment and solvent interaction and values fitted to the model equations well.

3.3.3 Effect of pretreatment on extraction kinetics

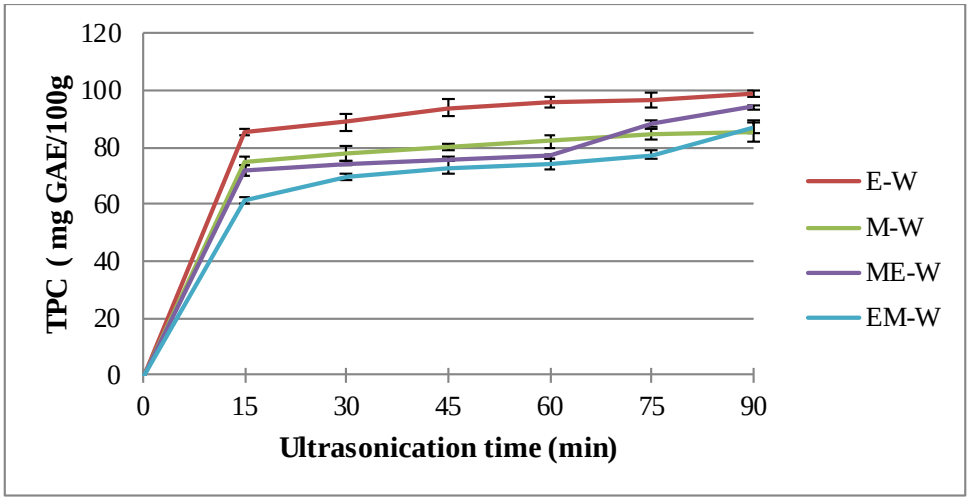
Pretreatment techniques significantly ($p \leq 0.05$) affect the amount of polyphenols in papaya peel extract and the level of polyphenols through each pretreatment was significantly increased with ultrasonication time. The experimental design resulted in varying total phenol yields depending on the type of pretreatment used. R2 value ranged from 0.811 to 0.985 in the water-ethanol extracts; between 0.966 to 0.997 in the ethanol extracts; and in water extract, R^2 value ranged from 0.987 to 0.999 (Table 3.4). High amounts of polyphenols were determined in the peel pretreated with enzymes in water extract (R^2 = 0.999) followed by microwave pretreatment (R^2 = 0.997), even though the amount of phenolics was higher in enzyme pretreatment for each solvent as compared with other pretreatments. Flavonoids are another biologically active group found in papaya. The significance of responses to different pretreatments was similar for the flavonoid extraction with maximum yield obtained by enzyme pretreatment with water as a solvent having R^2 value as 0.997, followed by microwave water having R^2 value as 0.996. Similarly, extracted with ethanol and water-ethanol solvents, the highest R^2 value was 0.985 and 0.977, obtained for enzyme pretreatment (Table 3.4). Apart from other pretreatment techniques, enzyme-microwave pretreatment extracted the least quantity of

phenolic compounds. This might be due to the deleterious effect of microwave on both the enzyme and phenolic compounds. As microwave produces energy in the form of heat which results in alteration in the structure of polyphenols as well as enzymes and thus reduces the specificity of enzyme. Another reason might be the development of an enzyme–polyphenol complex by hydrophobic interactions might result in poor efficiency of pretreatment methods for extract. Proteins have a certain number of locations where polyphenols can interact, notably at higher temperatures or after a pH change **[1]**. High temperatures, on the other hand, can lead the degradation of total polyphenols, particularly anthocyanins **[22]**.

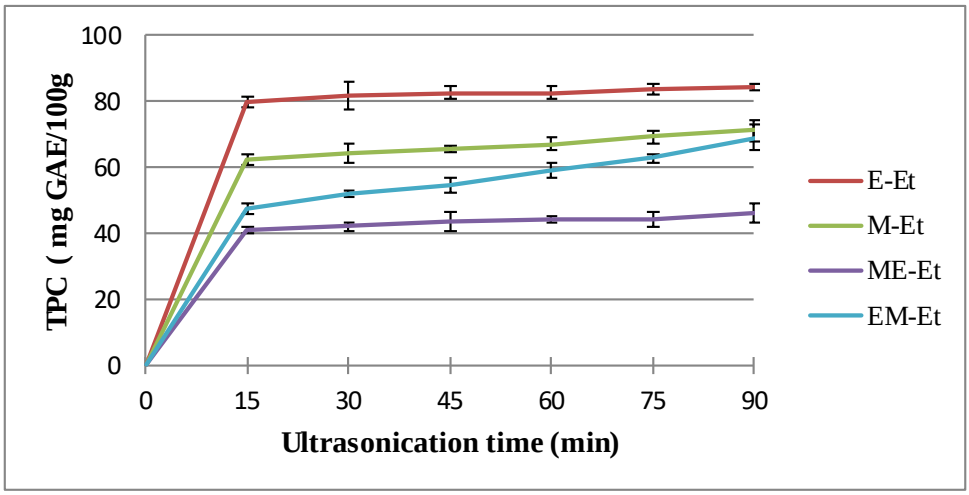
In this study, Table 3.6 also shows the kinetic study on antioxidant capacity obtained when samples were extracted using different pretreatments and results obtained were slightly different from phenolic content. A significantly higher R^2 was obtained in microwave pretreatment in each solvent followed by enzyme pretreatment. The maximum R^2 value was 0.999 in water solvent. This might be due to the components present in the extract being more water soluble. Water is a very stable solvent that exhibits selectivity for specific classes of compounds, which is due to the fact that the extraction process is dependent on the solvation of the target components in the liquid phase of the water. A similar behavior was observed with ethanol and water-ethanol solvent with highest R^2 as 0.999 and 0.999, as the greatest activity resulted in microwave pretreatment of papaya peel extract (Figure 3.3). In the case of EM and ME pretreatment, the antioxidant activity and phenolic content was not improved by the use of this combination of pretreatment.

Our findings support the use of enzymes as a viable technique for increasing the yield of phenolic components extracted from papaya peel. The quantity of phenolic compounds extracted by enzymes is affected by a number of factors, including enzyme type and concentration, pH, incubation temperature, and incubation duration. The Viscozyme L-assisted enzymatic pretreatment on papaya peel was investigated in this work, with an emphasis on identifying the released phenolic components. The considerably enhanced release of total phenolic compounds found in Viscozyme L. enzyme pretreatment compared to other pretreatment techniques clearly demonstrated that hydrolysis of cell-wall components of papaya peel increases phenolic compound extraction yields. This finding might be explained by enhanced cell-wall structure

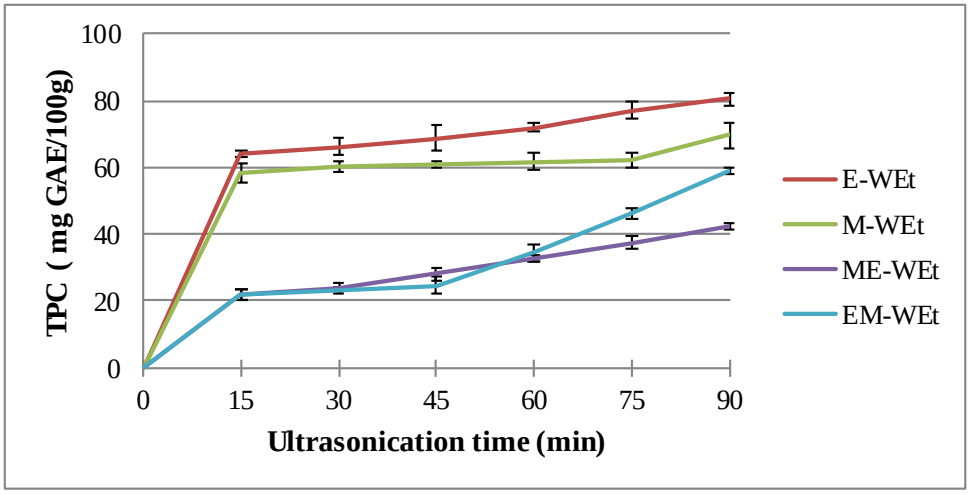
breakdown as a result of cell-wall component hydrolysis, particularly glycosidic bonds/linkages between phenolic compounds and cell-wall polysaccharides **[5,50]**. Furthermore, it is known that the breakdown of cell-wall polymers improves the permeability and porosity of plant cells, increasing the solubility of the cell's internal components and, as a result, increasing the concentration of phenolic compounds in extracts **[7,8]**. de Camargo et al. **[11]** discovered that Viscozyme, a commercial enzymatic combination, improved extraction of non-soluble compounds such as gallic acid, caffeic acid, and p-coumaric acid, as well as procyanidin dimers A and B, which were only detected in trace amounts or were completely missing in other extracts. Combining microwave pretreatments with enzymes did not lead to an increase in the release of total phenolic compounds ($p > 0.05$). The possible reason for this could be the high temperature and microwave radiation, which might have affected the extraction rate of phenolic compounds from the peel as well as enzymatic oxidation and polymerization processes. Microwave radiation can cause the structure of phenolic compounds to break down, leading to steric obstruction of enzyme binding sites to the substrate. This, in turn, inhibits the degradation of cell-wall components **[15,21,26].**



(a)

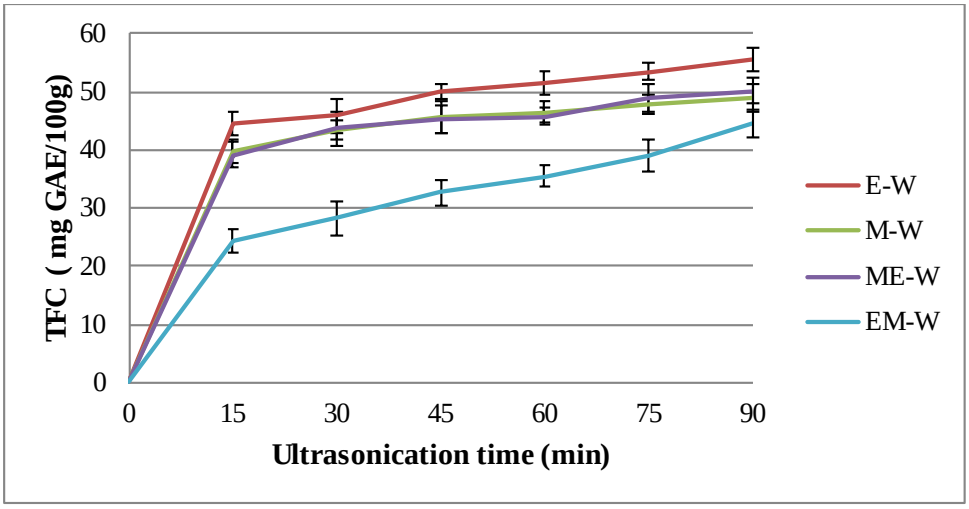


(b)

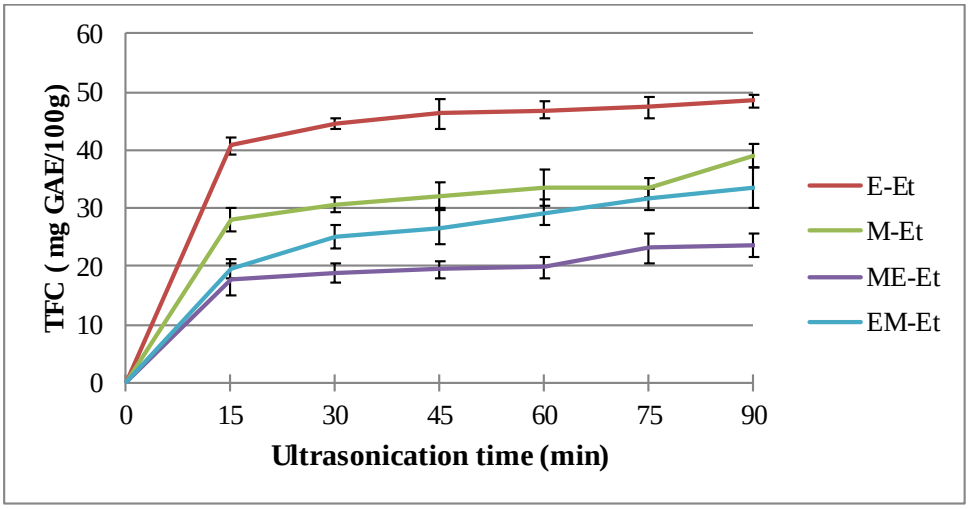


(c)

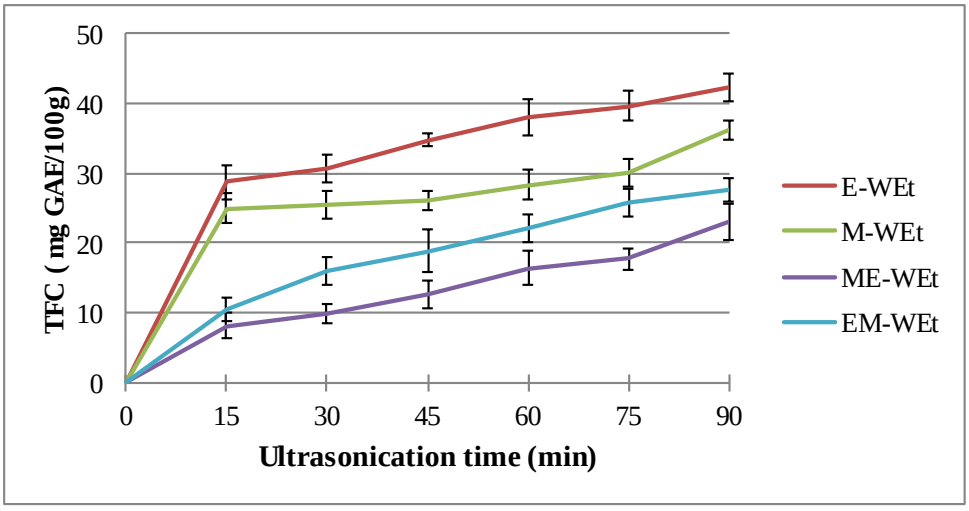
Figure 3.1: Effect of different pretreatment in different solvents on total phenolic content **(a)** Water **(b)** Ethanol **(c)** Water:Ethanol (1:1)



(a)

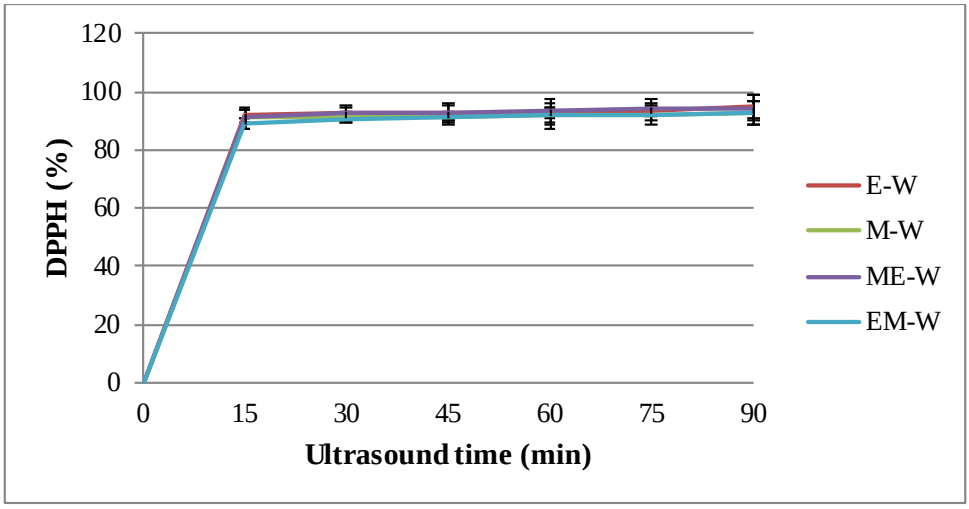


(b)

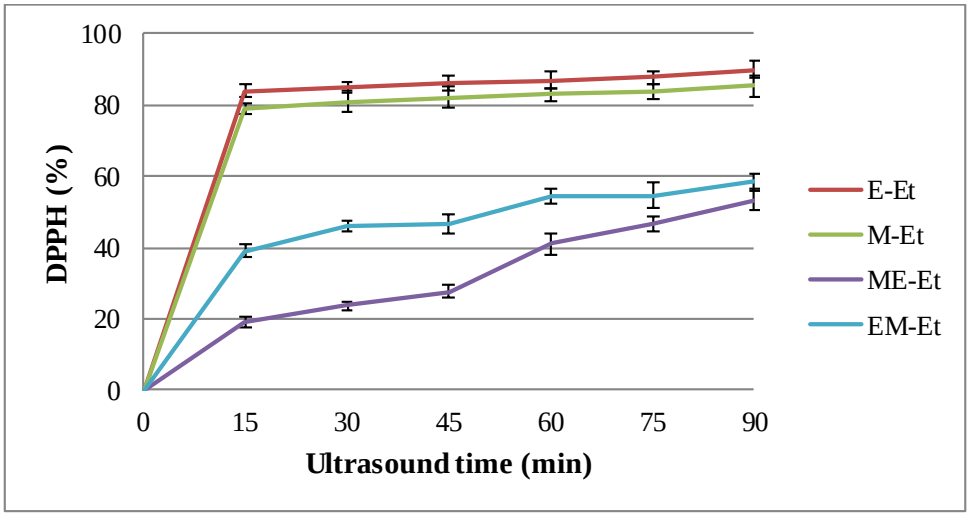


(c)

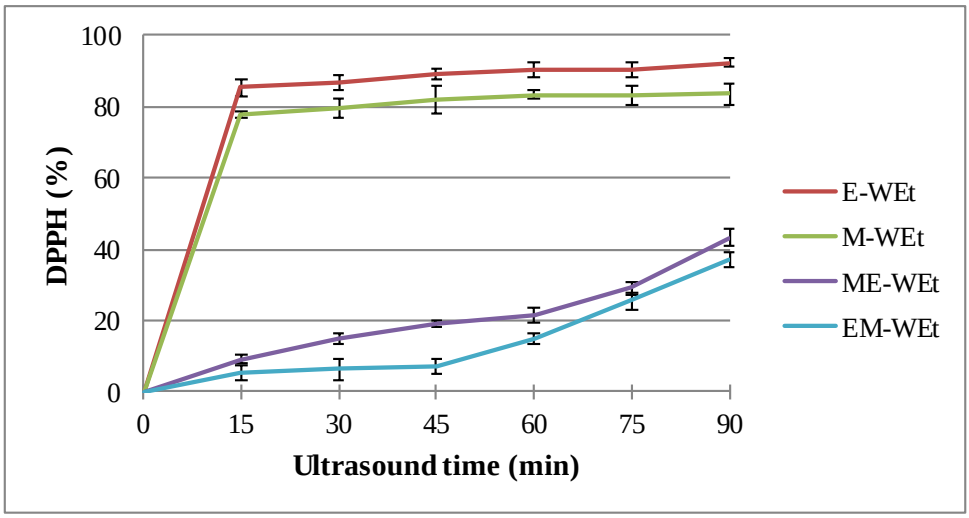
Figure 3.2: Effect of different pretreatment in different solvents on total flavonoid content (**a)** Water **(b)** Ethanol **(c**) Water:Ethanol (1:1)



(a)



(b)



(c)

Figure 3.3: Effect of different pretreatment in different solvents on total antioxidant activity (**a)** Water **(b)** Ethanol **(c**) Water:Ethanol (1:1)

3.3.4 Effect of solvent on extraction kinetics

Simultaneous experiments were performed to evaluate the influence of different solvents on the extracted TPC, TFC, and antioxidant activity of papaya peel samples by changing the contact surface between the different solvents and the papaya peel sample. The selection of appropriate extraction solvents is a critical step in parameter since it has a significant influence on extraction yield. The most difficult aspect of natural product extraction is predicting the interface between bioactive chemicals and extracting solvents owing to their varied biological structures. The choosing of solvent is mostly determined by the molecules to be extracted, as the composition of the extract and yield vary depending on the solvent. To explain the effects of different solvents with varying polarity on polyphenol extraction efficiency and antioxidant activity, total phenolic components were extracted using several techniques. As a result, various polarity solvents, such as ethanol, water, and water: ethanol (1:1), were employed as extracting solvents. In terms of extract concentration, mg GAE/100g, and extraction yield, the findings are shown in Figure 3.4.

Figure 3.4 shows the polyphenol concentration in the extract, and it was shown that water provided the best overall phenolic extraction having R^2 value of 0.999 followed by ethanol (R^2 = 0.997) in enzyme pretreatment. Similar results were obtained in microwave pretreatment, water showed the maximum yield of total phenolic compounds having R^2 value as 0.997 followed by ethanol (Table 3.4). Similarly, in microwave-enzyme pretreatment and enzyme-microwave pretreatment, water (R^2 = 0.995 and 0.987) showed better yield than other solvents.

Likewise, Table 3.5 shows the effects of different solvent applications in the extraction process on total flavonoid content from the papaya peel extract. It was observed that in enzyme pretreatment samples, a water solvent produced extracts with highest content of flavonoid (R^2 = 0.997) in comparison with the other studied solvents ($p < 0.05$). Significantly higher flavonoid content was obtained with water in microwave pretreatment as R^2 value was 0.996 compared with extracts obtained using ethanol and water: ethanol (Figure 3.5). A similar behavior was also observed in microwave-enzyme and enzyme-microwave pretreatment, as the highest extraction resulted from water treatment of papaya peel samples. In each case, it was observed that the TFC was improved by the use of water solvent extraction followed by ethanol.

Based on the DPPH results (Table 3.6), water was considered to be the best solvent for extracting antioxidant components from papaya peel, thus the values obtained using water were higher in all pretreatments and significantly different ($p < 0.05$) from all other values obtained by the DPPH analysis. Other investigations have found that water solvents absorb antioxidant and phenolic components from various natural sources more efficiently than other solvents **[26,49]**.

The solid–liquid extraction methods of antioxidants from pomegranate marc using water as a solvent were described using the second-order model by Qu et al. **[32]**. Using this method, it was revealed that in all cases, the solvent had the largest impact on the C_e, h, and k anticipated parameters. As a result, the second-order kinetic model may be used to explain extraction processes with a wide range of operating conditions, solvents, and ultrasound extraction parameters including extraction temperature, ultrasound amplitude level, pulse duration/interval ratio and solvent/solid ratio.

The increased activity of the enzyme (polyphenol oxidase) that destroys the phenolic compound in water–ethanol results in reduced extraction of total phenolic compounds, whereas these enzymes are ineffective in alcoholic medium. For the appropriate industrial uses, a proportional addition of water is more sustainable, cost-effective, and safe. In addition, it is less hazardous, less expensive, and more environmentally friendly than other extracting solvents. As a result, water was selected as the best solvent for the remaining experiments.

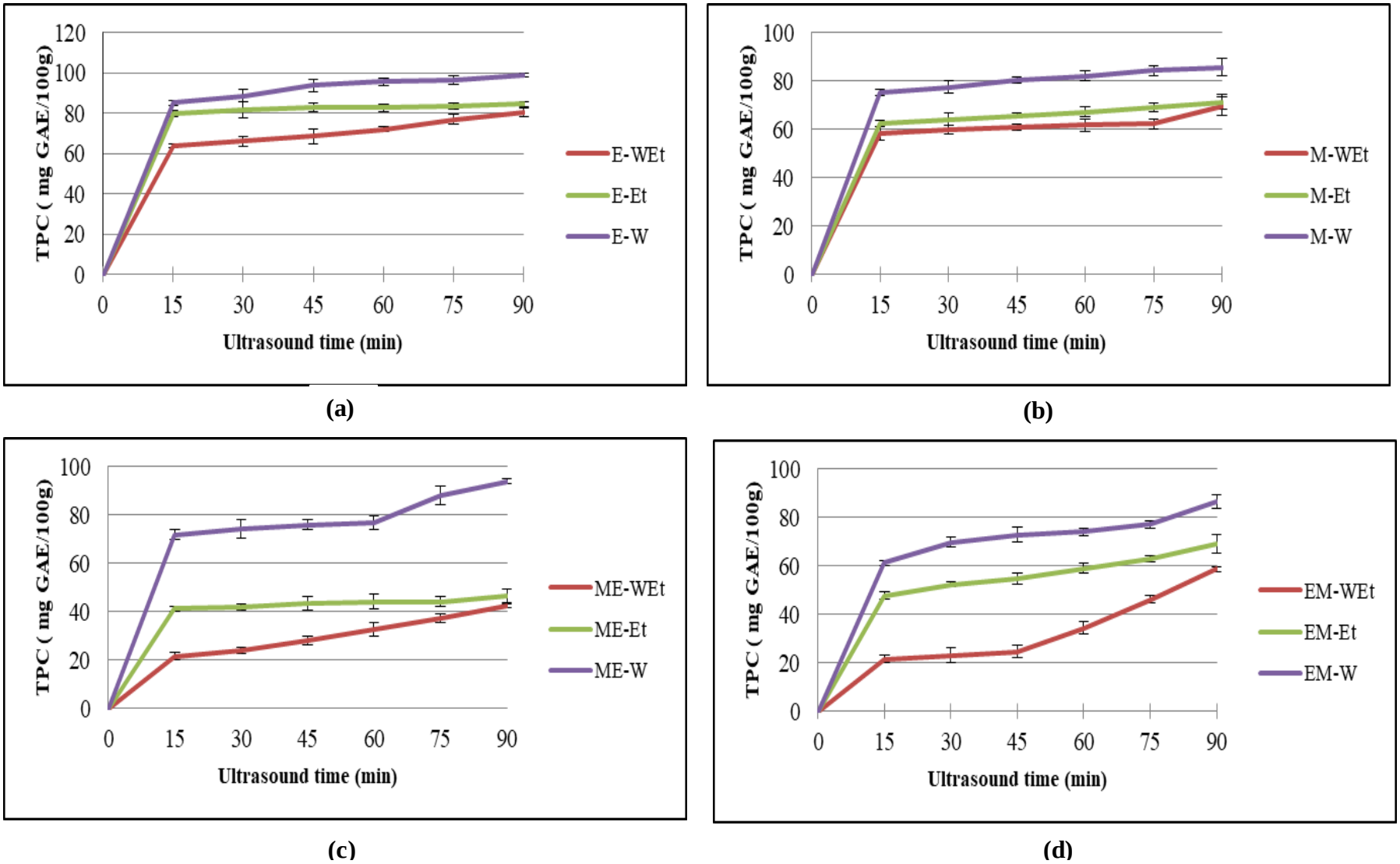


Figure 3.4: Effect of different solvents in different pretreatments on total phenolic content **(a)** Enzyme **(b)** Microwave **(c)** Microwave-Enzyme **(d)** Enzyme-Microwave

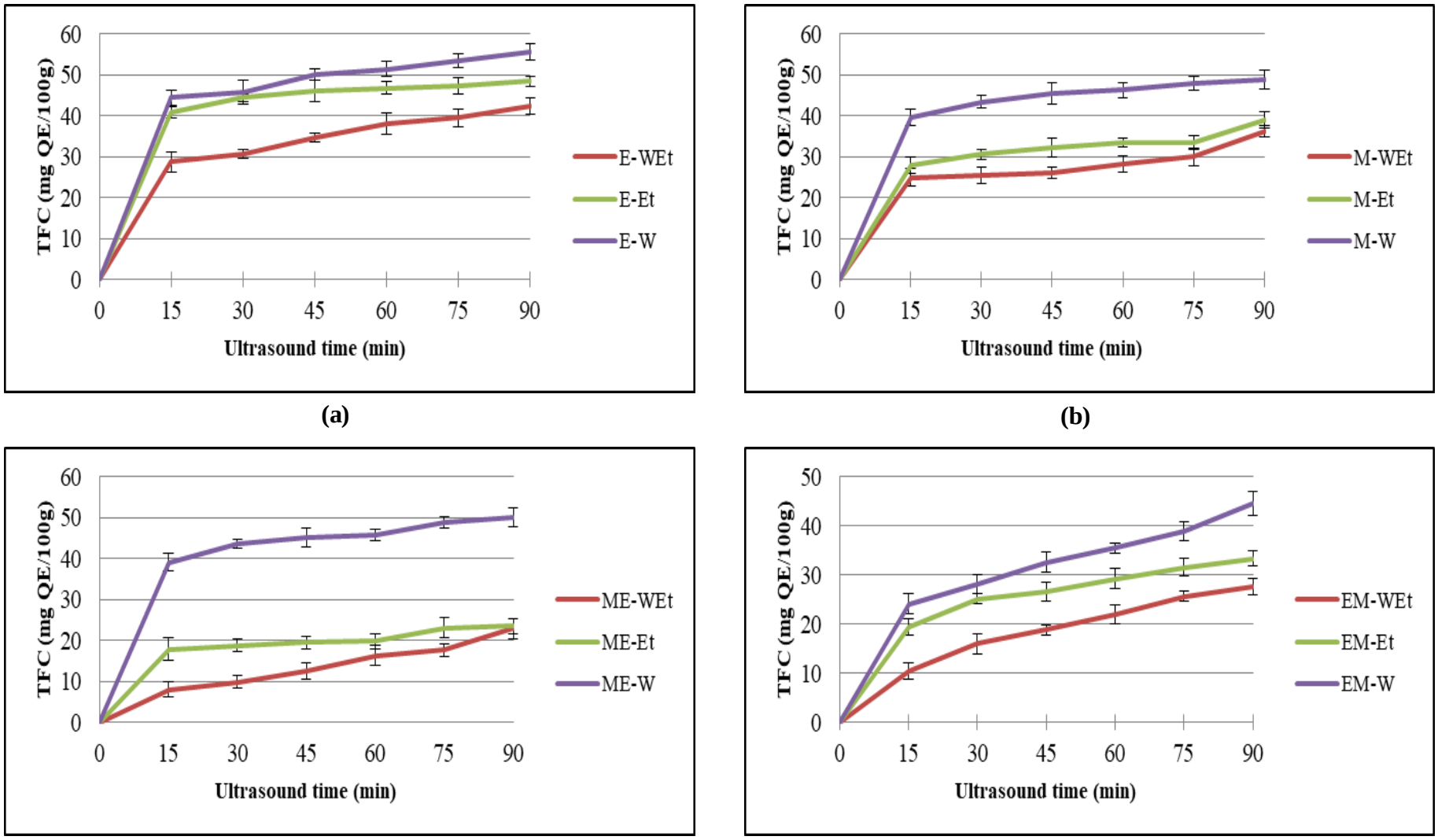


Figure 3.5: Effect of different solvents in different pretreatments on total flavonoid content **(a)** Enzyme **(b)** Microwave **(c)** Microwave-Enzyme **(d)** Enzyme-Microwave

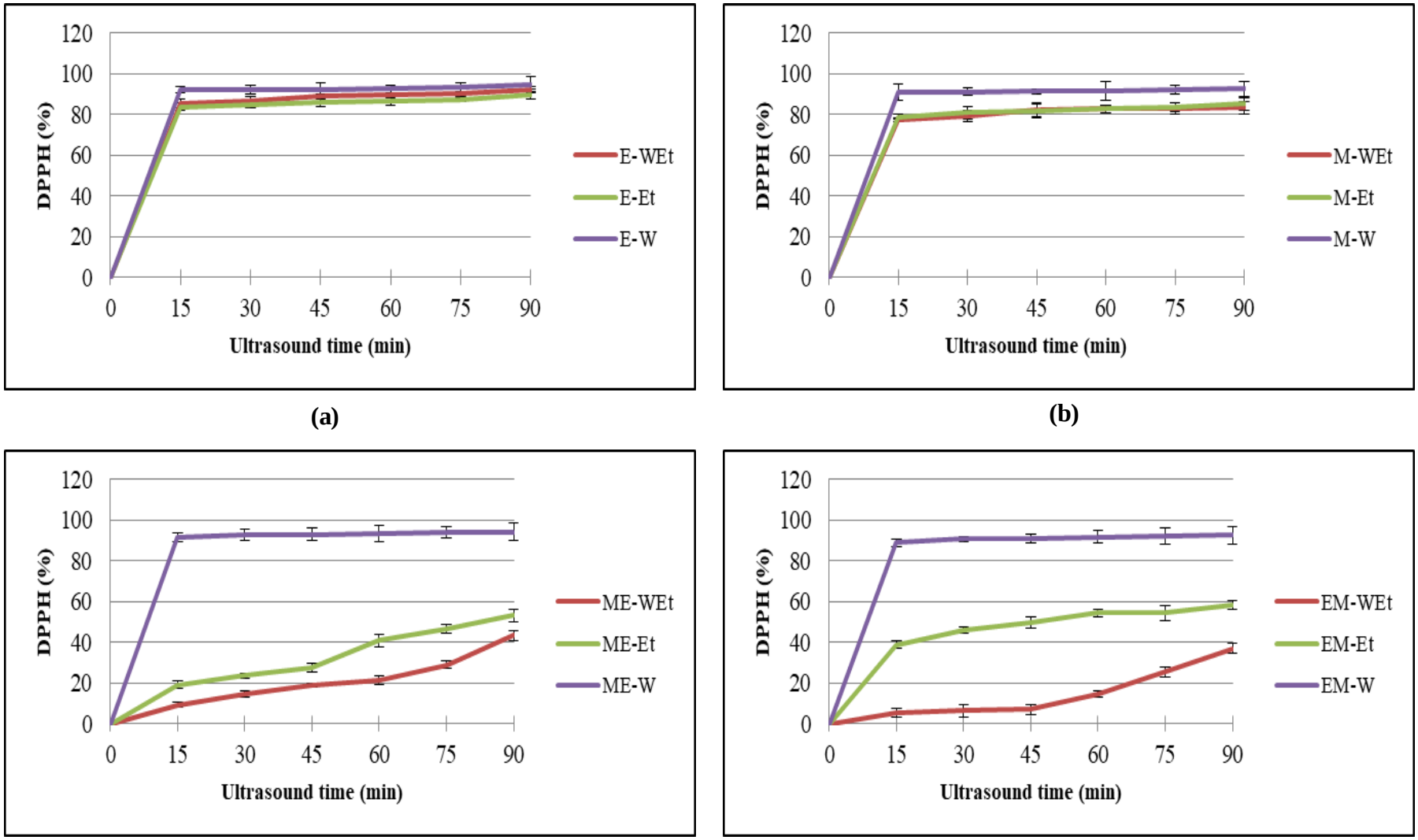


Figure 3.6: Effect of different solvents in different pretreatments on antioxidant activity **(a)** Enzyme **(b)** Microwave **(c)** Microwave-Enzyme **(d)** Enzyme-Microwave

3.3.5 Effect of ultrasound on extraction of phytochemicals and antioxidant activity

Ultrasound extraction of polyphenols from papaya peel was studied for different conditions of pretreatment and solvent interaction and compared with the non-ultrasonicated sample (the sample is not subjected to any additional ultrasound treatment). When comparing the extract yield, it could be seen that extracts obtained from the pretreated papaya peel samples by UAE and without UAE, a significant difference was noticed in the quantity of polyphenols ($p < 0.05$).

Figure 3.7 shows a comparison of the extraction graph obtained with and without ultrasonic assistance at various pretreatments and solvents. The ultrasonic irradiation had a substantial beneficial effect in all of the cases. Ultrasound, in reality, causes the creation of small bubbles that are exposed to rapid adiabatic compressions and expansions, causing local temperature and pressure rises **[18]**. As a result, the peels that were subjected to sonication may have contributed to the increased yields seen with ultrasound assistance. Toma et al. **[42]** found that ultrasounds had a substantial impact on the swelling of dried sample. Indeed, tissues that have been sonicated absorb more solvent. The cavitation process causes cell swelling, solvent absorption, and the expansion of cell wall pores during sonication, allowing for more diffusivity across cell walls. The increased extraction yields observed with ultrasonic assist might possibly be attributable to the fact that ultrasonication may cause a collapse of cell walls, allowing the cell content to be washed out more easily **[46]**. When the yields after 90 minutes are compared in Figure 3.7, Figure 3.8, and Figure 3.9, it can be concluded that ultrasound assisted extraction with varied pretreatments and solvent interaction with papaya peel may be as efficient as extraction without ultrasound and under identical conditions.

Eventually, the use of ultrasound assisted in the reduction of extraction time. In all of the extraction experiments, 15 minutes of ultrasound assistance resulted in higher extraction yields than 90 minutes without ultrasound. Time and energy reductions are essential in the manufacturing of natural extracts. Diouf et al. **[12]** proved that ultrasonic assisted extraction is a “environmentally friendly” extraction method as compared to conventional maceration by monitoring the CO_2 levels rejected in the atmosphere during polyphenol extraction from yellow birch.

The impact of ultrasonic assistance computed at the end of experimental runs (after 90 minutes) at various studied parameters is summarized in Figure 3.7. Equation 1 was used to determine the ultrasonic effect.

The effect of ultrasound on several pretreatment-solvent interactions on the extraction yields of papaya peel extract ranged from 8.07% to 72.95%, with the highest improvement of around 72.95% obtained in total phenolic content (Table 3.7) with flavonoids in the range of 19.11% to 77.99% (Table 3.8) and antioxidants in range of 2.49% to 90.42% (Table3.9), respectively.

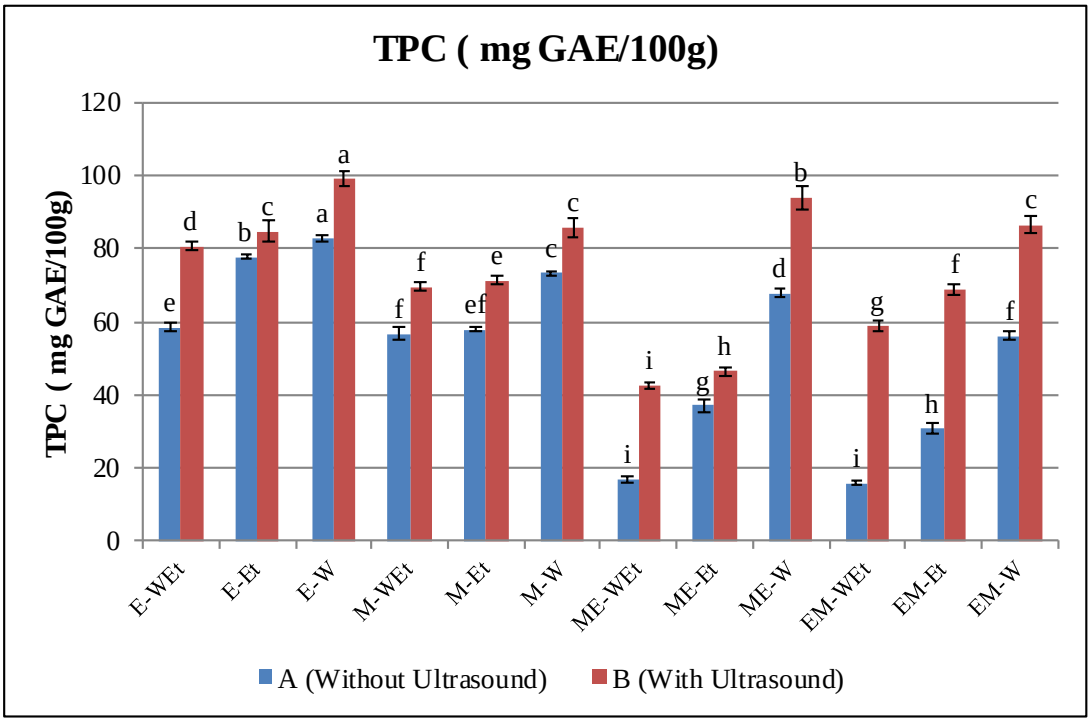


Figure 3.7: Effect of ultrasound on total phenolic content

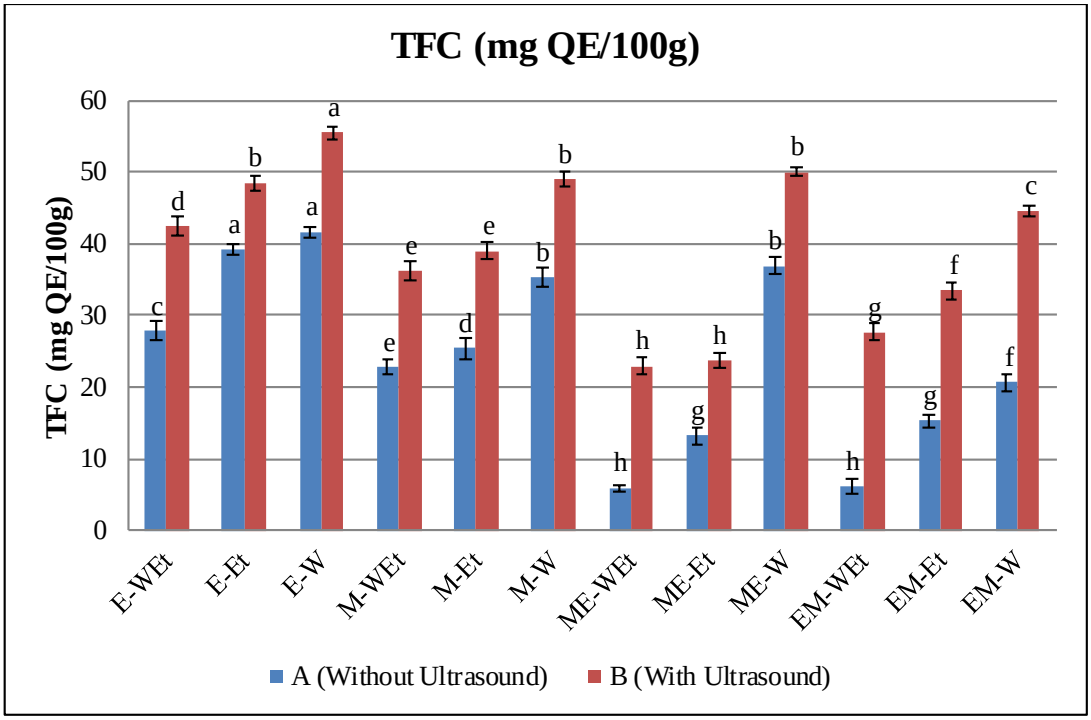


Figure 3.8: Effect of ultrasound on total flavonoid content

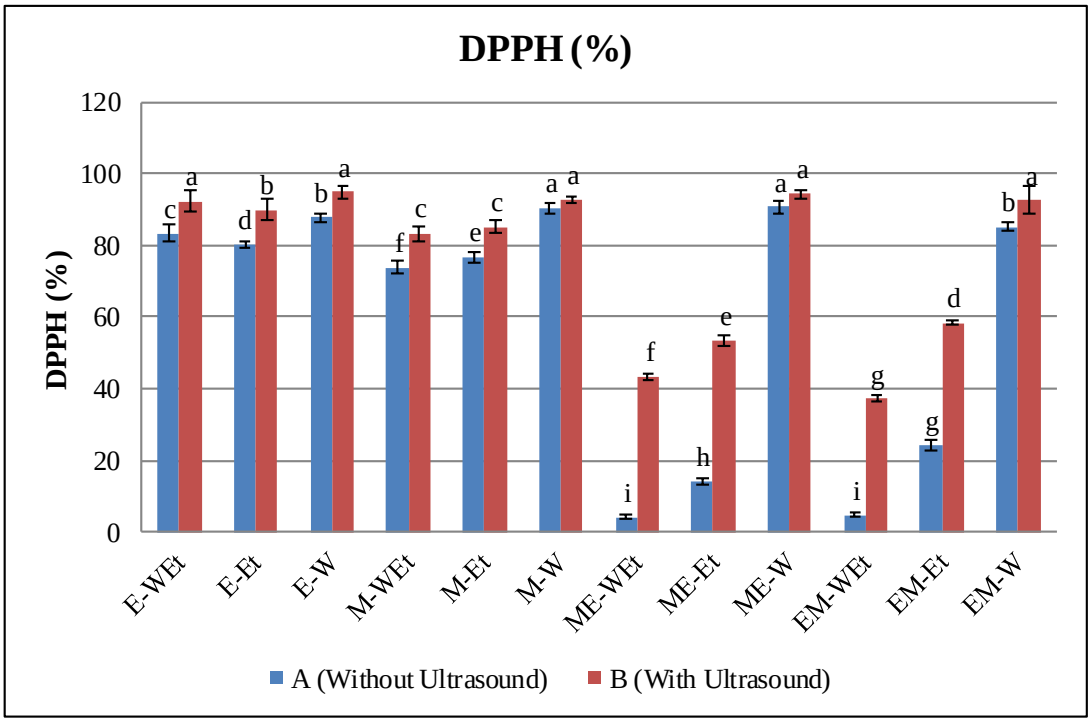


Figure 3.9: Effect of ultrasound on antioxidant activity

In general, it can be seen (Table 3.7) that the quantities of total polyphenols in the enzyme pretreatment samples are significantly higher than in other pretreated samples. Enzyme pretreatment enhances the effectiveness of extracting phenolic compounds,

while the different ratios of enzymes affect the polarity of the solvent and the solubility of the extracted compounds. In all of the studies, extraction yield was strongly time dependent, increasing with longer ultrasonic durations, particularly from 0 to 30 minutes, but more slowly from 30 to 90 minutes. As a result, the most effective extraction time for obtaining maximal polyphenol production was around 30 minutes. This phenomenon occurs because the extraction process can be divided into two distinct stages: the initial stage, which is rapid, involves the penetration of the solvent into the cellular structure and the dissolution of soluble components, while the subsequent stage, which is slower, involves the external diffusion of dissolved constituents through the porous structure of solid residues.

The majority of the extracts produced with ultrasonic assistance had significant antioxidant activity, which was related to the high phenolic contents found in these extracts (Table 3.9). Lower outcomes were seen mostly in situations when ultrasound was not used. The effective concentration as well as the correlation between antioxidant activity and polyphenol content in extracts generated under various conditions (pretreatment, solvent, time, ultrasound assistance or not) could be determined due to sufficient dilutions of the acquired native extracts. The content of polyphenols and the corresponding antioxidant activity were shown to have a strong relationship (Figure 3.9).

Table 3.7: Effect of ultrasound on extraction of total phenolic content

Treatments	Without Ultrasound (A)	With Ultrasound (B)	% = B-A/B*100
E-WEt	58.45 ± 0.98^{e}	80.58 ± 1.01^{d}	27.46
E-Et	77.73 ± 0.72^{b}	84.57 ± 2.98^{c}	8.07
E-W	82.79 ± 1.12^{a}	98.90 ± 2.1^{a}	16.28
M-WEt	56.63 ± 1.73^{f}	69.54 ± 1.09^{f}	18.57
M-Et	57.95 ± 0.50^{ef}	71.32 ± 1.21^{e}	18.74
M-W	73.23 ± 0.70^{c}	85.69 ± 2.25^{c}	14.54
ME-WEt	16.89 ± 0.74^{i}	42.56 ± 0.93^{i}	60.30
ME-Et	37.04 ± 1.55^{g}	46.37 ± 1.05^{h}	20.11
ME-W	67.87 ± 1.40^{d}	93.98 ± 3.16^{b}	27.78
EM-WEt	15.88 ± 0.71^{i}	58.73 ± 1.54^{g}	72.95
EM-Et	30.64 ± 1.41^{h}	68.93 ± 1.45^{f}	55.53
EM-W	56.11 ± 1.25^{f}	86.49 ± 2.09^{c}	35.12

values are presented as mean ± standard deviations.

Table 3.8: Effect of ultrasound on extraction of total flavonoid content

Treatments	Without Ultrasound (A)	With Ultrasound (B)	% = B-A/B*100
E-WEt	27.76 ± 1.21^{c}	42.34 ± 1.35^{d}	34.44
E-Et	39.19 ± 0.75^{a}	48.45 ± 1.00^{b}	19.11
E-W	41.67 ± 0.76^{a}	55.53 ± 0.90^{a}	24.96
M-WEt	22.76 ± 1.02^{e}	36.19 ± 1.39^{e}	37.10
M-Et	25.38 ± 1.49^{d}	38.98 ± 1.17^{e}	34.89
M-W	35.32 ± 1.33^{b}	48.91 ± 1.02^{b}	27.78
ME-WEt	5.91 ± 0.41^{h}	22.94 ± 1.13^{h}	74.22
ME-Et	13.1 ± 81.25^{g}	23.75 ± 1.02^{h}	44.47
ME-W	36.93 ± 1.09^{b}	50.04 ± 0.62^{b}	26.21
EM-WEt	6.08 ± 0.96^{h}	27.64 ± 1.16^{g}	77.99
EM-Et	15.33 ± 0.86^{g}	33.40 ± 1.06^{f}	54.09
EM-W	20.67 ± 1.17^{f}	44.55 ± 0.70^{c}	53.58

values are presented as mean ± standard deviations.

Table 3.9: Effect of ultrasound on extraction of antioxidant activity

Treatments	Without Ultrasound (A)	With Ultrasound (B)	% = B-A/B*100
E-WEt	83.30 ± 2.33^{c}	92.33 ± 2.74^{a}	9.78
E-Et	80.08 ± 1.06^{d}	89.91 ± 2.98^{b}	10.93
E-W	87.71 ± 1.02^{b}	94.79 ± 1.58^{a}	7.46
M-WEt	73.88 ± 1.61^{f}	83.42 ± 2.07^{c}	11.43
M-Et	76.77 ± 1.47^{e}	85.21 ± 1.66^{c}	9.90
M-W	90.37 ± 1.40^{a}	92.68 ± 1.15^{a}	2.49
ME-WEt	4.14 ± 0.53^{i}	43.29 ± 1.07^{f}	90.42
ME-Et	14.08 ± 0.91^{h}	53.27 ± 1.51^{e}	73.55
ME-W	90.60 ± 2.00^{a}	94.37 ± 1.27^{a}	3.99
EM-WEt	4.74 ± 0.63^{i}	37.08 ± 0.94^{g}	87.20
EM-Et	24.11 ± 1.49^{g}	58.33 ± 0.72^{d}	58.66
EM-W	85.13 ± 1.09^{b}	92.56 ± 3.87^{a}	8.02

values are presented as mean ± standard deviations.

Ultrasonication assists in the breakdown of pomegranate peel cell walls, releasing polyphenols and making the substrate more available to the enzyme **[34]**. Longer sonication times result in greater breakdown of cell components and increase in the diffusion of polyphenols into the solvent **[2,50]**. D' Alessandro et al. **[10]** also found that 15 minutes of ultrasound treatment improved polyphenol extraction yields from black chokeberry compared to 60 minutes of extraction without ultrasound. The extraction

process is influenced by both the ultrasonic energy and the amplitude level. The mass transfer process is facilitated by high ultrasonic power, which results in a slight increase in recovered phenolics **[14,48]**. According to Zhang et al. **[52]**, the impact of ultrasound is most prominent within the initial 30 minutes of extraction, as observed in their study on flaxseed oil using ultrasound-assisted extraction. They attributed this observation to the ability of ultrasonic vibrations to disrupt cell walls, leading to increased contact between the solvent and the substance, resulting in higher oil extraction on the surface. However, as the distance between inner cell walls increases, the effect of ultrasound diminishes. Consequently, ultrasonic waves significantly influence the rate of mass transfer during the stage of solvent penetration. When Pan et al. **[29]** applied ultrasonication in continuous and pulsed modes to extract antioxidants from dried pomegranate marc peels, they obtained a similar result. Vinatoru et al. **[47]** obtained a similar result when they used ultrasonic energy to extract bioactive components from carrot powder. Apart from comparing the two methods, ultrasound assisted extraction enhanced global extraction yields by 26%. In this situation, the lower the temperature and the greater the ultrasonic irradiation, the more antioxidants are soluble. According to Soria & Villamiel **[38]**, sonication improves the mass transfer of solutes in the solvent, improving antioxidant compound extraction.

The comparison reveals a significant improvement in extraction, which may be attributed to ultrasonic cavitation, since this is the only treatment variable that changes between the two experiments. The ultrasound extracts include more phenolic compounds than the extracts that were not treated with ultrasound. The antioxidant activities of polyphenols recovered under various circumstances were fairly comparable, suggesting that the extracts had a similar composition but differed in quantity.

3.3.6 HPLC analysis of extracted phytochemical extract

The extracted phenolic compound from E-W pretreated papaya peel was subjected to HPLC analysis to identify and quantify specific phenolic compounds. The retention times of the detected peaks were compared to those of pure compounds to isolate and identify nine phenolic compounds, including gallic acid, caffeic acid, syringic acid, ferulic acid, salicylic acid, rutin, p-coumaric acid, quercetin, and kaempferol (Table 3.10). The peaks of these compounds were detected at 280 nm and compared with standards to confirm their identity. The dominant compound identified in the extract was

rutin. The study conducted by Saeed et al. **[34]** also found the presence of phenolic compounds such as ferulic acid, p-coumaric acid, and caffeic acid in papaya skin. HPLC analysis verified that phytochemical extract from papaya peel can be used for the development of food product.

Table 3.10: HPLC analysis of papaya peel extract

Peak	Compounds	Retention time	Amount (mg/g)
1	Gallic acid	3.811	18.004
2	Caffeic acid	11.086	0.345
3	Syringic acid	12.101	42.975
4	Ferulic acid	14.901	5.553
5	Salicylic acid	15.059	23.913
6	Rutin	16.537	256.45
7	p-Coumaric acid	17.053	16.666
8	Quercetin	18.987	4.186
9	Kaempferol	20.518	34.535

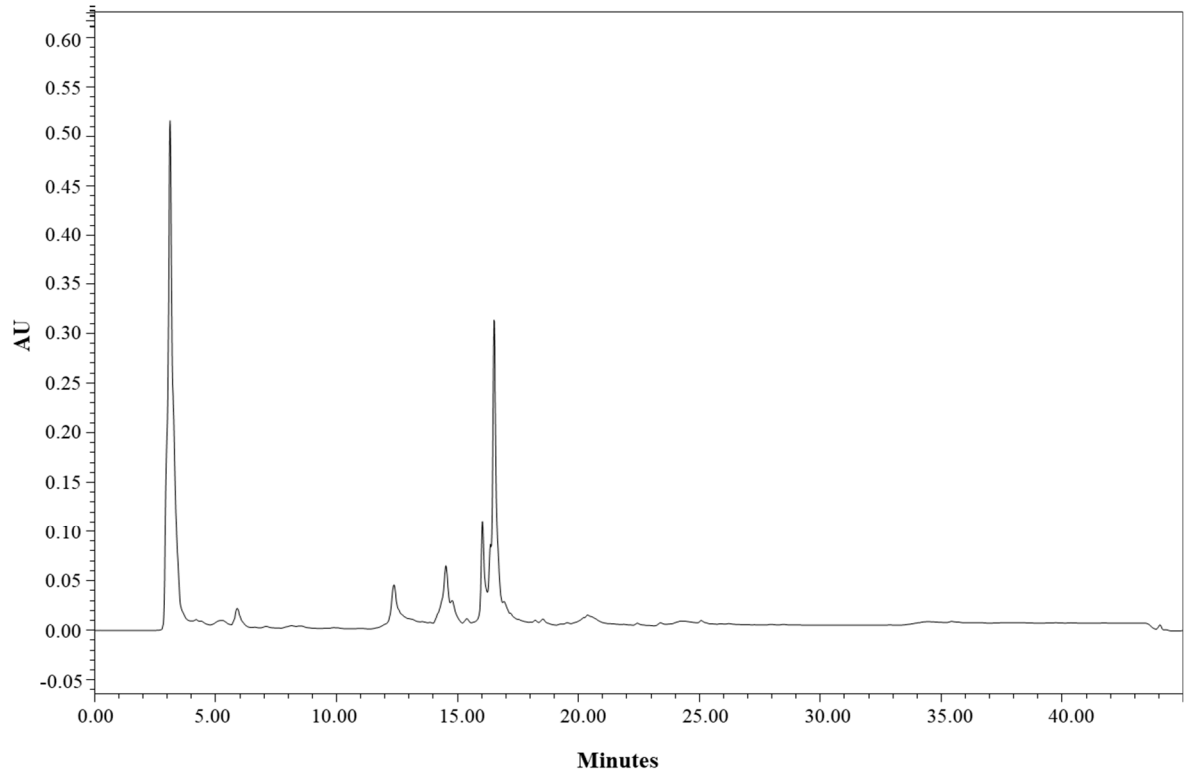


Figure 3.10: HPLC analysis of papaya peel phenolic extract

3.4 References

1. Aguilar, T., Loyola, C., de Bruijn, J., Bustamante, L., Vergara, C., von Baer, D., ... & Serra, I. Effect of thermomaceration and enzymatic maceration on phenolic compounds of grape must enriched by grape pomace, vine leaves and canes. *European Food Research and Technology*, 242(7), 1149–1158, 2016.
2. Al-Dhabi, N. A., Ponmurugan, K., & Jeganathan, P. M. Development and validation of ultrasound-assisted solid-liquid extraction of phenolic compounds from waste spent coffee grounds. *Ultrasonics Sonochemistry*, 34, 206–213, 2017.
3. AOAC. Determination of moisture, ash, protein and fat. *Official Method of Analysis of the Association of Analytical Chemists*, 141–144, 2005.
4. Ayala-Zavala, J. F., Rosas-Domínguez, C., Vega-Vega, V., & González-Aguilar, G. A. Antioxidant enrichment and antimicrobial protection of fresh-cut fruits using their own byproducts: Looking for integral exploitation. *Journal of Food Science*, 75(8), R175–R181, 2010.
5. Bagger-Jørgensen, R., & Meyer, A. S. Effects of different enzymatic pre-press maceration treatments on the release of phenols into blackcurrant juice. *European Food Research and Technology*, 219(6), 620–629, 2004.
6. Bhosale, P., & Udachan, I. S. Studies on utilization of Papaya peel and seed powder for development of fiber enriched functional cookies. *IJRAR-International Journal of Research and Analytical Reviews (IJRAR)*, 5(4), 459–466, 2018.
7. Cerda, A., Martínez, M. E., Soto, C., Poirrier, P., Perez-Correa, J. R., Vergara-Salinas, J. R., & Zúñiga, M. E. The enhancement of antioxidant compounds extracted from Thymus vulgaris using enzymes and the effect of extracting solvent. *Food Chemistry*, 139(1-4), 138–143, 2013.
8. Chen, S., Xing, X. H., Huang, J. J., & Xu, M. S. Enzyme-assisted extraction of flavonoids from Ginkgo biloba leaves: improvement effect of flavonol transglycosylation catalyzed by Penicillium decumbens cellulase. *Enzyme and Microbial Technology*, 48(1), 100–105, 2011.
9. D'Alessandro, L. G., Dimitrov, K., Vauchel, P., & Nikov, I. Kinetics of ultrasound assisted extraction of anthocyanins from Aronia melanocarpa (black chokeberry) wastes. *Chemical Engineering Research and Design*, 92(10), 1818–1826, 2014.
10. d'Alessandro, L. G., Kriaa, K., Nikov, I., & Dimitrov, K. Ultrasound assisted extraction of polyphenols from black chokeberry. *Separation and Purification Technology*, 93, 42–47, 2012.

11. de Camargo, A. C., Regitano-d'Arce, M. A. B., Biasoto, A. C. T., & Shahidi, F. Enzyme-assisted extraction of phenolics from winemaking by-products: Antioxidant potential and inhibition of alpha-glucosidase and lipase activities. *Food Chemistry*, 212, 395–402, 2016.
12. Diouf, P. N., Stevanovic, T., & Boutin, Y. The effect of extraction process on polyphenol content, triterpene composition and bioactivity of yellow birch (Betula alleghaniensis Britton) extracts. *Industrial Crops and Products*, 30(2), 297–303, 2009.
13. do Nascimento, A. D. P., Soares, L. A. L., Stragevitch, L., & Danielski, L. Extraction of Acrocomia intumescens Drude oil with supercritical carbon dioxide: Process modeling and comparison with organic solvent extractions. *The Journal of Supercritical Fluids*, 111, 1–7, 2016.
14. Dotto, J. M., & Abihudi, S. A. Nutraceutical value of Carica papaya: A review. *Scientific African*, 13, e00933, 2021.
15. Food and Agriculture Organization (FAO) of the United Nations. *FAOSTAT Database*. Retrieved from http://www.fao.org/faostat/en/#home, 2021.
16. Goula, A. M. Ultrasound-assisted extraction of pomegranate seed oil–Kinetic modeling. *Journal of Food Engineering*, 117(4), 492–498, 2013.
17. Huynh, N. T., Smagghe, G., Gonzales, G. B., Van Camp, J., & Raes, K. Enzyme-assisted extraction enhancing the phenolic release from cauliflower (Brassica oleracea L. var. botrytis) outer leaves. *Journal of Agricultural and Food Chemistry*, 62(30), 7468–7476, 2014.
18. Ikram, E. H. K., Stanley, R., Netzel, M., & Fanning, K. Phytochemicals of papaya and its traditional health and culinary uses–A review. *Journal of Food Composition and Analysis*, 41, 201–211, 2015.
19. Jamal, P., Akbar, I., Jaswir, I., & Zuhanis, Y. Quantification of Total Phenolic compounds in Papaya fruit peel. *Pertanika Journal of Tropical Agricultural Science*, 40(1), 2017.
20. Japón-Luján, R., Luque-Rodríguez, J. M., & De Castro, M. L. Dynamic ultrasound-assisted extraction of oleuropein and related biophenols from olive leaves. *Journal of Chromatography A*, 1108(1), 76–82, 2006.
21. Jayaprakasha, G. K., Singh, R. P., & Sakariah, K. K. Antioxidant activity of grape seed (Vitis vinifera) extracts on peroxidation models in vitro. *Food Chemistry*, 73(3), 285–290, 2001.

22. Kammerer, D., Claus, A., Schieber, A., & Carle, R. A novel process for the recovery of polyphenols from grape (Vitis vinifera L.) pomace. *Journal of Food Science*, 70(2), C157–C163, 2005.
23. Kapasakalidis, P. G., Rastall, R. A., & Gordon, M. H. Effect of a cellulase treatment on extraction of antioxidant phenols from black currant (Ribes nigrum L.) pomace. *Journal of Agricultural and Food Chemistry*, 57(10), 4342–4351, 2009.
24. Kulkarni, A. P., & Aradhya, S. M. Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chemistry*, 93(2), 319–324, 2005.
25. Martial-Didier, A. K., Hubert, K. K., Parfait, K. E. J., & Kablan, T. Phytochemical properties and proximate composition of papaya (Carica papaya L. var solo 8) peels. *Turkish Journal of Agriculture-Food Science and Technology*, 5(6), 676–680, 2017.
26. Martins, S., Aguilar, C. N., Garza-Rodriguez, I. D. L., Mussatto, S. I., & Teixeira, J. A. Kinetic study of nordihydroguaiaretic acid recovery from Larrea tridentata by microwave-assisted extraction. *Journal of Chemical Technology & Biotechnology*, 85(8), 1142–1147, 2010.
27. Murthy, T. P. K., & Manohar, B. Mathematical modeling of supercritical carbon dioxide extraction kinetics of bioactive compounds from mango ginger (Curcuma amada Roxb). *Open Journal of Organic Chemistry*, 2, 36–40, 2014.
28. Norsker, M., Bloch, L., & Adler-Nissen, J. Enzymatic degradation of plant cell wall polysaccharides: the kinetic effect of competitive adsorption. *Food/Nahrung*, 43(5), 307–310, 1999.
29. Pan, Z., Qu, W., Ma, H., Atungulu, G. G., & McHugh, T. H. Continuous and pulsed ultrasound-assisted extractions of antioxidants from pomegranate peel. *Ultrasonics Sonochemistry*, 19(2), 365–372, 2012.
30. Parveen, J., Iqrah, A., Yumi, Z., & Irwandi, J. Process development for maximum lycopene production from selected fruit waste and its antioxidant and antiradical activity. *Journal of Food Processing and Technology*, 7(4), 2016.
31. Pathak, P. D., Mandavgane, S. A., and Kulkarni, B. D. Waste to wealth: a case study of papaya peel. *Waste and Biomass Valorization*, 10(6):1755–1766, 2019.
32. Qu, W., Pan, Z., & Ma, H. Extraction modeling and activities of antioxidants from pomegranate marc. *Journal of Food Engineering*, 99(1), 16–23, 2010.

33. Rakotondramasy-Rabesiaka, L., Havet, J. L., Porte, C., & Fauduet, H. Solid–liquid extraction of protopine from Fumaria officinalis L.—Kinetic modelling of influential parameters. *Industrial Crops and Products*, 29(2-3), 516–523, 2009.
34. Saeed, F., Arshad, M. U., Pasha, I., Naz, R., Batool, R., Khan, A. A., ... & Shafique, B. Nutritional and phyto-therapeutic potential of papaya (Carica papaya Linn.): an overview. *International Journal of Food Properties*, 17(7), 1637–1653, 2014.
35. Saikia, S., Mahnot, N. K., & Mahanta, C. L. Optimisation of phenolic extraction from Averrhoa carambola pomace by response surface methodology and its microencapsulation by spray and freeze drying. *Food Chemistry*, 171, 144–152, 2015.
36. Singleton, V. L., & Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158, 1965.
37. Sit, N., Deka, S. C., & Misra, S. Combined effect of ultrasound and enzymatic pretreatment on yield and functional properties of taro (Colocasia esculenta) starch. *Starch-Stärke*, 66(11-12), 959–967, 2014.
38. Soria, A. C., & Villamiel, M. Effect of ultrasound on the technological properties and bioactivity of food: a review. *Trends in Food Science & Technology*, 21(7), 323–331, 2010.
39. Spiro, M., & Siddique, S. Kinetics and equilibria of tea infusion: kinetics of extraction of theaflavins, thearubigins and caffeine from Koonsong broken pekoe. *Journal of the Science of Food and Agriculture*, 32(11), 1135–1139, 1981.
40. Sun-Waterhouse, D., Wen, I., Wibisono, R., Melton, L. D., & Wadhwa, S. Evaluation of the extraction efficiency for polyphenol extracts from by-products of green kiwifruit juicing. *International Journal of Food Science & Technology*, 44(12), 2644–2652, 2009.
41. Tao, Y., Zhang, Z., & Sun, D. W. Experimental and modeling studies of ultrasound-assisted release of phenolics from oak chips into model wine. *Ultrasonics Sonochemistry*, 21(5), 1839–1848, 2014.
42. Toma, M., Vinatoru, M., Paniwnyk, L., & Mason, T. J. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrasonics Sonochemistry*, 8(2), 137–142, 2001.
43. Veličković, D. T., Milenović, D. M., Ristić, M. S., & Veljković, V. B. Kinetics of ultrasonic extraction of extractive substances from garden (Salvia officinalis L.) and

glutinous (Salvia glutinosa L.) sage. *Ultrasonics Sonochemistry*, 13(2), 150–156, 2006.

44. Vetal, M. D., Lade, V. G., & Rathod, V. K. Extraction of ursolic acid from Ocimum sanctum by ultrasound: process intensification and kinetic studies. *Chemical Engineering and Processing: Process Intensification*, 69, 24–30, 2013.
45. Viacava, G. E., Ansorena, M. R., Roura, S. I., González-Aguilar, G. A., & Ayala-Zavala, J. F. Fruit processing byproducts as a source of natural antifungal compounds. *Antifungal Metabolites from Plants*, 447–461, 2013.
46. Vinatoru, M. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrasonics Sonochemistry*, 8(3), 303–313, 2001.
47. Vinatoru, M., Toma, M., Radu, O., Filip, P. I., Lazurca, D., & Mason, T. J. The use of ultrasound for the extraction of bioactive principles from plant materials. *Ultrasonics Sonochemistry*, 4(2), 135–139, 1997.
48. Wang, X., Wu, Y., Chen, G., Yue, W., Liang, Q., & Wu, Q. Optimisation of ultrasound assisted extraction of phenolic compounds from Sparganii rhizoma with response surface methodology. *Ultrasonics Sonochemistry*, 20(3), 846–854, 2013.
49. Wijekoon, M. J. O., Bhat, R., & Karim, A. A. Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (Etlingera elatior Jack.) inflorescence. *Journal of Food Composition and Analysis*, 24(4-5), 615–619, 2011.
50. Yadav, G., Singh, A., Bhattacharya, P., Yuvraj, J., & Banerjee, R. Comparative analysis of solid-state bioprocessing and enzymatic treatment of finger millet for mobilization of bound phenolics. *Bioprocess and Biosystems Engineering*, 36(11), 1563–1569, 2013.
51. Yogiraj, V., Goyal, P. K., Chauhan, C. S., Goyal, A., & Vyas, B. Carica papaya Linn: an overview. *International Journal of Herbal Medicine*, 2(5), 01–08, 2014.
52. Zhang, Z. S., Wang, L. J., Li, D., Jiao, S. S., Chen, X. D., & Mao, Z. H. Ultrasound-assisted extraction of oil from flaxseed. *Separation and Purification Technology*, 62(1), 192–198, 2008.
53. Zhu, C. P., Zhai, X. C., Li, L. Q., Wu, X. X., & Li, B. Response surface optimization of ultrasound-assisted polysaccharides extraction from pomegranate peel. *Food Chemistry*, 177, 139–146, 2015.