# Ultrasound Extraction of Valonea Tannin and its Effects on Extraction Yield

*by*

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## Abstract

Ultrasound extraction of valonea tannin from the acorn cups of the Quercus species was investigated and compared to the conventional extraction method. Effects of ultrasonic power, time and temperature on extraction were examined. Considering the highest extraction yields and tannin contents of two extraction methods, it was found out that ultrasound extraction result in 17.16% increase in extraction yield and 6.61% in tannin amount. Additionally, apart from the temperature study, the sound effect on extraction yields that occurred during ultrasound extractions was also determined. The results were evaluated statistically by using One-Way ANOVA and Duncan tests with the SPSS 15 statistic program. The results showed that ultrasonic extraction method can be used as an alternative to conventional method for extraction of tannin (valonea) from acorn cups.

## Resumen

Extracción por ultrasonido del tanino de las cápsulas de bellotas de la valonea de la especie Quercus fueron investigadas y comparadas con el método convencional de extracción. Los efectos de la energía ultrasónica, el tiempo y la temperatura sobre la extracción fueron examinados. Teniendo en cuenta los mayores rendimientos de extracción y el contenido de taninos de los dos métodos de extracción, se encontró que el resultado de la extracción por ultrasonido aumenta 17,16% el rendimiento de la extracción y 6,61% la cantidad de tanino. Además, aparte del estudio de la temperatura, el efecto del sonido en los rendimientos de extracción producidos durante las extracciones ultrasónicas fue también determinado. Los resultados se evaluaron estadísticamente mediante el uso de los ensayos One-Way ANOVA y Duncan con el programa estadístico SPSS 15. Los resultados mostraron que el método de extracción por ultrasonido podría ser utilizado como una alternativa al método convencional para la extracción de taninos (valonea) de las cápsulas de bellotas.

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## INTRODUCTION

Vegetable tannins are one of the many types of secondary compounds found in plants. They occur in one form or another in a substantial part of the Plant Kingdom especially in higher plants and in particular families of dicotyledons, the most notable being perhaps the Leguminosae (e.g. Acacia sp.), Anacardiaceae (eg. sumach, quebracho), Combretaceae (eg. myrobalan), Rhizophoraceae (eg. mangrove), Myrtaceae (eg. eucalyptus) and Polygonaceae (eg. canaigre). Although almost all plants contain tannins, only few species have sufficient amounts to be of commercial importance. Many of the commercially most significant tannin materials such as wattle, mangrove, quebracho and myrobalan originate in tropical or sub-tropical climes although plants rich in tannin also occur in temperate zones.[1]

Tannins are deposited in various parts of plants such as barks, woods, fruits, pods, leaves and roots. Conventionally, tannins are stripped out from tannin rich plant materials by hot water extraction which is conducted within big, open or closed vessels by subjecting the plant materials to hot water.

Extraction of phenolic compounds from plant materials is influenced by their chemical nature, the employed extraction method, particle size of the plant material, storage time and extraction conditions.[2] The main purposes in tannin extraction from plant materials are maximum extraction of water soluble materials and to recover them with minimum losses from the final extract. In extraction processes mainly the temperature, the quality and amount of water and processing time have great influence on extraction yield, concentration and quality of tannin. Therefore, extraction conditions should be optimized in terms of productivity and quality by considering the chemical structure and properties of the tannin.

There is a growing demand for developing suitable extraction techniques for more efficient and effective extraction of available active matters from the plant materials.[3] Different techniques as supercritical fluid extraction (SFE) – using either pure or modified $CO_2$ [4] – and microwave-assisted extraction[5] have been applied. These techniques offer a better control over the extraction conditions and allow the extraction to be performed in shorten times and in a more selective way. However, ultrasound-assisted leaching is an effective way to extract analytes from different matrices in shorter time than other extraction techniques.[6] It has become a good alternative extraction method when compared to classical extraction methods due to its high efficiency, low energy and water consumption (no reflux or refrigeration are needed). Besides, ultrasound-assisted extraction is a well-established method in the processing of plant material, particularly to extract low molecular weight substances.[7]

Because of these properties, recently ultrasonic extraction has been tested as an alternative method to extract tannins from plant materials.[8-12]

Sound waves with frequency above the human audible range of 16 KHz are called ultrasound.[13-15] It can be divided into three frequency ranges; power ultrasound (16-100 kHz), high frequency ultrasound (100 kHz-1 MHz) and diagnostic ultrasound (1-10 MHz).[16] The generally accepted explanation for ultrasound enhancement the effect of ultrasonic waves on the vegetal material breaks the cells and releases the cell's contents into extraction medium.[17,18] When a liquid is irritated by ultrasound, micro bubbles can appear, grow and oscillate extremely fast an even collapse violently if the acoustic pressure is high enough. These collapses occurring near a solid surface generate micro jets and shock waves. Moreover in the liquid phase surrounding the particles, high micro mixing increase the heat and mass transfer and even the diffusion of species inside the pores of the solid.[19,20] This happen is known as cavitation. The low frequency ultrasound (that is power ultrasound) generates large cavitation bubbles resulting in high temperatures and pressures in the cavitation zone. As the frequency increases the cavitation zone becomes less violent and in the MHz range no cavitation is observed anymore and the main mechanism is acoustic streaming. While medical imaging operates at frequencies in the MHz range, most industrial applications (processing of chemicals, food as well as cleaning) operate between 16 and 100 KHz because cavitation can be produced within this frequency range.[21] Ultrasonic bath and probe units are two common devices for ultrasonic application. Ultrasonic probes have the advantage over ultrasonic bath in that they focus their energy on a localized sample zone, thereby providing more efficient cavitation in the liquid.[22] The high intensity ultrasonic probe system can introduce a much greater intensity of ultrasound into the sample container than the ultrasonic bath (up to 100 times greater). Hence it can drastically accelerate the extraction of the analytes from solid samples.[23] Variations in the extraction yield for different plants may result from structure, rheological or compositional differences resulting in varying degrees of susceptibility to ultrasound shock waves and likelihood that cavitation bubble will contact with the plant surface causing micro-jetting.[24]

Valonea, which is the name of the tannin obtained from the fruits (acorn) of Quercus species (Quercus macrolepis) growing naturally in the west part of Turkey, has a great technical and economic importance for Turkish and World Leather Industry. It is one of the well-known vegetable tannins, used for tannage and retannage processes in leather making. Valonea tannin is obtained from tannin rich cups and beards of acorns by conventional hot water extraction. In order to investigate possible increase in extraction yields of this commercially important tannin, the present study is aimed on investigation and comparison of extraction yields of valonea by ultrasound and conventional methods.

## MATERIALS AND METHODS

### Material

Acorn cups were obtained from Tezcan Leather Company operating in Aydin/Turkey. They were crushed to 2-5mm pieces. In view of tannin contents of the cups and the beards of acorn cups are not the same, to obtain more consistent and reliable test results and to provide homogeneity of the samples, acorn cups were weighed 10.00 g, to be 4.00 g of beards and 6.00 g of cups for each extraction.

### Methods

***Determination of Moisture Content of Acorn Cups*** Moisture contents of the acorn cups were determined according to SLC 113-Determination of Moisture.[25] Experiments were replicated three times and average moisture content was determined.

***Determination of Maximum Extractable Material (MEM)***

Maximum extractable material from the acorn cups was determined according to procedure described by Sivakumar et.al[8] 10.00 grams of crushed and dried acorn cups were soaked in 300 ml of distilled water (I-float) in a clean glass beaker and maintained at 70 °C with magnetic stirring. The beaker was tightly covered with aluminum foil to prevent the evaporation of water from the beaker. Samples were taken every day after 8 h and the weight of dried extract was analyzed by following a gravimetric procedure. Likewise, extraction was performed using fresh II-float and III-float separately to extract maximum soluble material, and analysis was performed. Then total maximum soluble material per gram of the acorn cups used was calculated and taken as MEM.

***Extractions***

Acorn cups were crushed and dried before the extraction processes. Both conventional and ultrasonic extractions were performed by using distilled water as solvent, keeping the ratio of acorn cups to water as 1:30.

***Conventional Extraction of Acorn Cups***

For the conventional extraction experiments, 10.00 g of acorn cups were placed in a 500 ml beaker and then 300 ml of distilled water was added on the plant material. Acorn cups were subjected to conventional extraction processes under constant temperatures at 30, 50, 70 and 90°C for 4, 6 and 8h. There was no mechanical effect (stirring) during the extraction processes (as in practice). 2 ml of samples were taken from the extracts into clean, dried and weighed glass dishes, in order to determine amount of carried solid matter into extraction medium. The extracts were dried in a hot-air oven (100±2 °C) until all the water evaporated and only the solid matter was left. The dishes were then cooled in a desiccator and weighed, in this way, the amount of the solid matter was determined. The yield was calculated using the follow equation:

Yield= [(*Extract obtained (g)*/ Amount of acorns used (g)]*100

The extraction experiments were repeated three times.

***Ultrasonic Extraction of Acorn Cups***

Similarly with conventional method, for the ultrasonic extractions 10.00 g of acorn cups and 300 ml of distilled water were used. Ultrasonic extraction experiments were performed by using ultrasonic probe (BANDELIN SONOPULS, Germany, 20 kHz and 30-200 W). 40, 60, 80 and 100W ultrasonic powers were used in extractions without any external heating or cooling. The extractions were carried out for 4, 6 and 8 hours. The amount of solid matter in the extracts was determined in the same way with conventional method. All the experiments were done in triplicate.

***Tannin Analysis***

The tannin contents of the extracts were determined according to SLC 114, 115, 116 and 117 methods.[26-29] All of the analyses were carried out triplicate and average of analysis data was used in the graphs.

***Statistical Analysis***

The results were evaluated statistically by using One-Way ANOVA and Duncan tests at SPSS 15 statistic program. All the data were presented as mean for three independent measurements. Comparison of means was analyzed by Duncan test and differences were considered significant when $p< 0.05$.

## RESULTS AND DISCUSSION

***Moisture Content and Maximum Extractable Material (MEM) of Acorn Cups***

Average moisture content of the acorn cups was found to be 11.06%. Average maximum extractable material (MEM) from the acorn cups was found to be 49.80% which means that 0.498g of solid matter obtained from the extraction of each gram of the acorn cups.

***Extraction Yield and Tannin Content of Acorn Cups by Conventional Method***

Considering the data obtained from conventional extractions; as expected, it was seen that there is an increase in extraction yields in conjunction with increasing extraction temperature and time. While the increase of extraction temperature from 30°C to 90°C resulted in 2.37 to 3.05 times of extraction yields, the increase of extraction time from 4 to 8 h resulted in 1.11 to 1.42 extraction yields. In brief, it is possible to say that generally the increases in extraction time and especially extraction temperature have significant effect on extraction yield and caused an increase (Table I).

From the statistical evaluation of the effects of temperature and time on extraction yields; the differences between means

**TABLE I**
**Effects of extraction time and temperature on yields (%) in conventional method.**

| | | | Extraction Time | | |
|---|---|---|---|---|---|
| **Temperature** | **n** | | **4 hours** | **6 hours** | **8 hours** |
| 30°C | 3 | Min. | 15.80 | 21.90 | 22.01 |
| | | Max. | 15.95 | 22.33 | 23.76 |
| | | X±S.E.M | $15.90\pm0.05^{d,B}$ | $22.12\pm0.13^{d,A}$ | $22.60\pm0.58^{d,A}$ |
| 50°C | 3 | Min. | 28.57 | 33.06 | 34.70 |
| | | Max. | 29.44 | 33.79 | 36.98 |
| | | X±S.E.M | $29.10\pm0.27^{c,C}$ | $33.40\pm0.21^{c,B}$ | $35.83\pm0.66^{c,A}$ |
| 70°C | 3 | Min. | 33.64 | 40.75 | 42.34 |
| | | Max. | 34.51 | 42.34 | 43.94 |
| | | X±S.E.M | $33.93\pm0.29^{b,C}$ | $41.66\pm0.48^{b,B}$ | $43.21\pm0.47^{b,A}$ |
| 90°C | 3 | Min. | 48.43 | 52.64 | 52.35 |
| | | Max. | 48.58 | 52.78 | 54.52 |
| | | X±S.E.M | $48.53\pm0.05^{a,B}$ | $52.73\pm0.05^{a,A}$ | $53.55\pm0.64^{a,A}$ |

$^{a,b,c,d}$; Differences are important between means which are showed different letters at the same coloums
$^{A,B,C,D}$; Differences are important between means which are showed different letters at the same rows

of extraction yields were found to be significant ($p$<0.05) for all extraction times (4, 6, 8 h) and temperatures (30, 50, 70 and 90°C) (Table I).

All commercial vegetable tannins (the solids extracted from plant materials) consist of tannins, sugars, salts, gums, lignin and other components of plant materials passed into extracts. As the solid material extracted from the acorn cups are also not completely the tannins, we have investigated the percentage of tannins within the solid material. From the tannin analysis; it was seen that, as well as the amounts of solid material extracted from acorn cups were increasing with ascending temperatures and time, the percentage of tannin correspondingly increased till 70°C. The percentage of tannin within solid materials slightly decreased at 90°C - 8 h, as larger amounts of water soluble non-tannins passed into extract at this condition. The duration of extraction also contribute to this result (Table II).

While in 8 h extraction time the temperature has no significant effect (P>0.05) on the percentage of tannin within the extracted solids, in 4 and 6 hours extractions the temperature was found to be effective (P<0.05) (Table II). From the statistical evaluation of the effect of the time on the percentage of tannin; it was found to be important for the extraction which was conducted at 30°C ($p$<0.05) but, it was not important for 50, 70 and 90°C extractions ($p$>0.05) (Table II).

The effects of extraction temperature and time on amount of tannin (g) obtained by conventional extractions are given in Table III. Considering the table; all temperature and time changes provided statistically significant changes in amounts of tannins ($p$<0.05) in extractions, and increasing temperature and time caused an increase in amount of extracted tannin.

***Extraction Yield and Tannin Content of Acorn Cups by Ultrasonic Method***

Effect of ultrasonic power variation on extraction yield was aimed to be investigated by using 40, 60, 80, 100, 120 and 140 W ultrasonic powers without any external heating or cooling. During ultrasonic extraction trials it was noticed that the temperature of the solution is getting higher by increasing ultrasonic power. For this reason we have decided to monitor temperature variations in the extract depending on the ultrasonic powers, as the temperature is one of the most important parameter for extraction (Table IV).

From the measurements, it was seen that increase of ultrasonic power cause significant increase in ambient (extract solution) temperature. The temperatures of the extracts were approached to boiling temperature at 100W ultrasonic extraction. At this point the probe and also the device were overheated and the cavitation was considerably decreased. Lorimer and Mason has stated that; usually, as the temperature increases up 50°C extraction efficiency is increased as a result of the larger

**TABLE II**
**Effects of extraction time and temperature on % tannin in conventional method**

| | | | Extraction Time | | |
|---|---|---|---|---|---|
| **Temperature** | **n** | | **4 hours** | **6 hours** | **8 hours** |
| **30°C** | 3 | Min. | 51.80 | 57.24 | 59.11 |
| | | Max. | 57.47 | 65.60 | 66.77 |
| | | X±S.E.M | 53.85±1.82$^{c, B}$ | 60.92±2.46$^{b, AB}$ | 63.96±2.43$^{b, A}$ |
| **50°C** | 3 | Min. | 61.10 | 62.88 | 64.05 |
| | | Max. | 63.32 | 68.02 | 72.89 |
| | | X±S.E.M | 62.40±0.67$^{b, A}$ | 64.87±1.59$^{b, A}$ | 68.28±2.56$^{ab, A}$ |
| **70°C** | 3 | Min. | 67.33 | 70.90 | 69.60 |
| | | Max. | 70.08 | 72.13 | 74.41 |
| | | X±S.E.M | 68.68±0.80$^{a, A}$ | 71.66±0.38$^{a, A}$ | 72.04±1.39$^{a, A}$ |
| **90°C** | 3 | Min. | 69.81 | 69.29 | 69.64 |
| | | Max. | 71.79 | 72.34 | 71.78 |
| | | X±S.E.M | 70.97±0.60$^{a, A}$ | 70.86±0.88$^{a, A}$ | 70.45±0.67$^{ab, A}$ |

$^{a, b, c, d,}$ Differences are important between means which are showed different letters at the same coloums
$^{A, B, C; D,}$ Differences are important between means which are showed different letters at the same rows

number of cavitation nucleus formed.[21] As the temperature approaches the boiling point of the liquid, ineffective sonication occurs as a result of the decrease in surface tension and increase in vapor pressure within the micro bubble, which in turn causes the damping of the shock wave.[30] For this reason ultrasound extraction trails with 120 and 140 W decided to be repealed. The extraction yields of ultrasound extraction trials (without external heating or cooling) are given below (Table V).

When Table V is examined, we can clearly see that the extraction yield increase by increasing ultrasonic power and time. Increasing ultrasonic power from 40 to 100 W resulted in 1.43 to 1.76 fold of extraction yields. However, especially in higher ultrasonic powers (100 W), extraction time has not resulted significant increase in extraction yields (1.07 fold). The difference between the means of the extraction yields obtained by varying ultrasonic powers was found to be statistically important ($P<0.05$) for each extraction times. From the statistical consideration of the yield variation depending on the extraction time; by contrast with the 100 W, the difference between means of yield at 40, 60 and 80W ultrasonic extractions were found to be important ($p<0.05$) (Table V).

Percentages of tannins within the solid matters extracted from acorn cups are given in Table VI. Increasing extraction time at 40 and 60 W ultrasonic powers caused an increase in tannin content of the extract. However, increasing amounts of non-tannins extracted from plant materials at 80 and especially at 100W ultrasonic powers diminished the percentage of tannins within the extracted total solids. This situation can be explained that the rate of tannin in the extracted total solids decrease due to increased amount of non-tannin matters extracted from plant materials. Also, it was seen that at the end of 100W ultrasonic power and 8 hours extraction time conditions, while the extracted total solid matter was increased 14.6%, the tannin percentage in total solids was decreased 4.48% according to the 80W - 8h extraction conditions. From the statistical evaluation of the effect of ultrasonic power and extraction time on percentage of tannin within total solids; it was concluded that neither ultrasonic power nor extraction time (for both; $p > 0.05$) has effect on the percentage of tannin in ultrasonic extractions.

Statistical analysis regarding the amounts of tannins revealed that; the increase in ultrasonic power has resulted statistically significant ($P<0.05$ for all extraction times) changes in amounts of extracted tannins in all extraction times and the difference between the amounts of tannins in 40, 60 and 80 W extractions were statistically important ($p<0.05$) for all extraction times, which means; in 40, 60 and 80 W ultrasonic extractions, increasing extraction time increases the amount of extracted tannin. Conversely, the extracted amount of tannin was not changing ($P>0.05$) depending on time at 100 W (Table VII).

## TABLE III
## Effects of extraction time and temperature on amount of tannin (g) in conventional method.

| | | | Extraction Time | | |
|---|---|---|---|---|---|
| **Temperature** | **n** | | **4 hours** | **6 hours** | **8 hours** |
| **30°C** | 3 | Min. | 8.26 | 12.53 | 13.01 |
| | | Max. | 9.08 | 14.52 | 15.86 |
| | | X±S.E.M | $8.56\pm0.26^{d, B}$ | $13.48\pm0.58^{d, A}$ | $14.47\pm0.83^{d, A}$ |
| **50°C** | 3 | Min. | 17.45 | 20.97 | 22.22 |
| | | Max. | 18.55 | 22.98 | 26.11 |
| | | X±S.E.M | $18.16\pm0.35^{c, C}$ | $21.67\pm0.65^{c, B}$ | $24.48\pm1.16^{c, A}$ |
| **70°C** | 3 | Min. | 22.65 | 30.22 | 30.18 |
| | | Max. | 23.69 | 35.98 | 31.68 |
| | | X±S.E.M | $23.30\pm0.33^{b, B}$ | $32.22\pm1.88^{b, A}$ | $31.12\pm0.47^{b, A}$ |
| **90°C** | 3 | Min. | 33.91 | 36.47 | 37.46 |
| | | Max. | 34.77 | 38.18 | 38.13 |
| | | X±S.E.M | $34.44\pm0.27^{a, B}$ | $37.36\pm0.49^{a, A}$ | $37.72\pm0.21^{a, A}$ |

$^{a, b, c, d,}$ Differences are important between means which are showed different letters at the same coloums
$^{A, B, C; D,}$ Differences are important between means which are showed different letters at the same rows

## TABLE IV
## Temperature variations in extracts depending on ultrasonic power and time.

| **Time** | **Ultrasonic Power** | | | |
|---|---|---|---|---|
| | **40 W** | **60 W** | **80 W** | **100 W** |
| **1h** | 50 °C | 68°C | 86°C | 88°C |
| **2h** | 63°C | 78°C | 90°C | 92°C |
| **3h** | 70°C | 83°C | 92°C | 98°C |
| **4h** | 72°C | 83°C | 94°C | 98°C |
| **5h** | 72°C | 86°C | 94°C | 98°C |
| **6h** | 72°C | 86°C | 94°C | 98°C |
| **7h** | 76°C | 88°C | 94°C | 98°C |
| **8h** | 76°C | 88°C | 94°C | 98°C |

The comparison of extraction yield (%), % tannin and amount of tannin (g) obtained by conventional and ultrasonic extraction methods are given in Table VIII. From the comparison of ultrasonic and conventional methods in terms of highest yield and amount of tannin: The highest yields of conventional (90°C–8h) and ultrasound (100W–8h) extractions were found to be 53.6% and 62.8%. Which means the yield was increased 17.16% by ultrasound extraction. Furthermore the highest yield of conventional method could be achieved in less than 4h by 100W ultrasonic extraction. The highest amounts of tannins obtained by conventional and ultrasound extraction methods were found to be 37.8g (90°C for 8h) and 40.3g (100W for 6h) respectively. By ultrasound, the amount of tannin extracted from acorn cups were increased 6.61% in comparison with conventional method. Additionally, the MEM could be achieved in 4-6 h by conventional (at 90°C) and in less than 4 h by ultrasound (at 100W) methods.

Additionally, in order to investigate only the sound effect on extraction yield, the temperatures were kept constant at 30, 50 and 70°C in ultrasound extractions. Since the ambient temperature was increased and could not be kept constant at 90°C, the cavitation was considerably decreased, the probe and also the device were overheated. For this reason the experiments at this temperature were repealed.

## TABLE V

**Effects of extraction time and power on yields (%) in ultrasonic method.**

| | | | Extraction Time | | |
|---|---|---|---|---|---|
| **Temperature** | **n** | | **4 hours** | **6 hours** | **8 hours** |
| **40W** | 3 | Min. | 32.63 | 39.88 | 42.63 |
| | | Max. | 33.93 | 41.62 | 45.39 |
| | | X±S.E.M | 33.21±0.38$^{d, C}$ | 40.50±0.56$^{d, B}$ | 43.89±0.80$^{d, A}$ |
| **60W** | 3 | Min. | 42.34 | 45.10 | 47.27 |
| | | Max. | 44.81 | 48.00 | 49.74 |
| | | X±S.E.M | 43.16±0.82$^{c, B}$ | 46.45±0.84$^{c, A}$ | 48.58±0.72$^{c, A}$ |
| **80W** | 3 | Min. | 45.10 | 49.01 | 54.09 |
| | | Max. | 47.42 | 53.51 | 55.68 |
| | | X±S.E.M | 46.01±0.71$^{b, C}$ | 51.38±1.30$^{b, B}$ | 54.76±0.48$^{b, A}$ |
| **100W** | 3 | Min. | 58.29 | 59.74 | 60.03 |
| | | Max. | 58.87 | 61.34 | 66.27 |
| | | X±S.E.M | 58.58±0.17$^{a, B}$ | 60.37±0.49$^{a, AB}$ | 62.83±1.83$^{a, A}$ |

$^{a, b, c, d,}$ Differences are important between means which are showed different letters at the same coloums
$^{A, B, C; D,}$ Differences are important between means which are showed different letters at the same rows

## TABLE VI

**Effects of extraction time and power on (%) tannin in ultrasonic method.**

| | | | Extraction Time | | |
|---|---|---|---|---|---|
| **Temperature** | **n** | | **4 hours** | **6 hours** | **8 hours** |
| **40W** | 3 | Min. | 64.19 | 62.78 | 65.35 |
| | | Max. | 66.07 | 67.35 | 71.92 |
| | | X±S.E.M | 65.10±0.54$^{a, A}$ | 65.79±1.51$^{a, A}$ | 69.00±1.93$^{a, A}$ |
| **60W** | 3 | Min. | 65.50 | 66.13 | 67.74 |
| | | Max. | 67.82 | 71.10 | 70.89 |
| | | X±S.E.M | 66.91±0.72$^{a, A}$ | 68.48±1.44$^{a, A}$ | 69.09±0.94$^{a, A}$ |
| **80W** | 3 | Min. | 64.81 | 67.40 | 65.12 |
| | | Max. | 67.95 | 68.78 | 69.39 |
| | | X±S.E.M | 65.95±1.00$^{a, A}$ | 68.17±0.41$^{a, A}$ | 67.03±1.26$^{ab, A}$ |
| **100W** | 3 | Min. | 66.23 | 64.00 | 63.66 |
| | | Max. | 68.14 | 69.07 | 64.36 |
| | | X±S.E.M | 67.47±0.62$^{a, A}$ | 66.79±1.49$^{a, AB}$ | 64.05±0.20$^{b, B}$ |

$^{a, b, c, d,}$ Differences are important between means which are showed different letters at the same coloums
$^{A, B, C; D,}$ Differences are important between means which are showed different letters at the same rows

## TABLE VII

**Effects of extraction time and power on amount of tannin (g) in ultrasonic method.**

| | | | Extraction Time | | |
|---|---|---|---|---|---|
| **Temperature** | **n** | | **4 hours** | **6 hours** | **8 hours** |
| **40W** | 3 | Min. | 21.22 | 25.03 | 28.52 |
| | | Max. | 21.84 | 28.03 | 31.65 |
| | | X±S.E.M | $21.61\pm0.20^{c, C}$ | $26.66\pm0.87^{d, B}$ | $30.28\pm0.92^{b, A}$ |
| **60W** | 3 | Min. | 28.55 | 29.82 | 32.02 |
| | | Max. | 29.35 | 32.89 | 34.54 |
| | | X±S.E.M | $28.87\pm0.24^{b, B}$ | $31.82\pm1.00^{c, A}$ | $33.57\pm0.78^{b, A}$ |
| **80W** | 3 | Min. | 29.23 | 33.49 | 35.22 |
| | | Max. | 32.22 | 36.06 | 40.72 |
| | | X±S.E.M | $30.36\pm0.94^{b, B}$ | $35.02\pm0.78^{b, A}$ | $37.41\pm1.68^{a, A}$ |
| **100W** | 3 | Min. | 38.80 | 39.25 | 38.63 |
| | | Max. | 40.05 | 41.46 | 42.18 |
| | | X±S.E.M | $39.52\pm0.37^{a, A}$ | $40.31\pm0.64^{a, A}$ | $40.24\pm1.04^{a, A}$ |

[a, b, c, d,] Differences are important between means which are showed different letters at the same coloums
[A, B, C; D,] Differences are important between means which are showed different letters at the same rows

The yields obtained by ultrasound extraction at constant temperatures are given in Table IX.

From the comparison of ultrasonic extraction yields with conventional extractions at constant temperatures, it was seen that only the sound effect apart from temperature contribution results in 5.07 – 27.93% at 30°C, 21.10 – 25.13% at 50°C and 7.15 - 23.31% at 70°C increase in extraction yields in itself.

## CONCLUSIONS

From conventional extraction trials, it was seen that increasing temperature had significant effect on extraction yield. However, while increasing temperature from 70°C to 90°C improved the yield, the increasing amounts of non-tannins passed into the extract resulted a slight decrease of the percentage of tannin within the extracted materials (from 72.0% to 70.5%).

As for the ultrasound extraction; it was observed that ultrasonic power was very effective for extraction yield. But, it was noticed that the temperature of the extraction liquor gradually increased correspondingly with the ultrasonic power and as the temperature was approached the boiling point of the liquid, ineffective sonication occurred. For this reason, the highest ultrasonic power for extractions was selected to be 100 W. At the end of 6 h extraction with 100 W ultrasonic power, the highest amount of tannin (40.3g) could be obtained with 60.4% extraction yield and 66.8 percentage of tannin. Although prolonging the extraction time from 6 h to 8 h resulted an increase in extraction yield, the percentage of tannin and also the amount of tannin within the extract was not improved. This is attributed to increasing amounts of non-tannins passed into the extract and possible fragmentation of tannins due to the extraction conditions in time.

From the comparison of the highest extraction yields and tannin contents of two extraction methods; it was found out that ultrasound extraction result in 17.16% increase in extraction yield and 6.61% in tannin amount. Furthermore the highest yield of conventional method could be achieved in less than 4 h by 100 W ultrasonic extractions. Additionally, apart from the temperature, the sound effect on extraction yields that occurred during ultrasound extractions was also determined. As a result, the use of ultrasound extraction method enabled an increase in extraction yield, and amount of tannin (valonea) extracted from acorn cups. Also it seems possible to decrease extraction time by this method.

## TABLE VIII
## Comparison of ultrasonic and conventional extraction methods.

| | Extraction Time | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 4h | | | 6h | | | 8h | | |
| Temperature | Extraction Yield (%) | Tannin Content (%) | Tannin Amount (g) | Extraction Yield (%) | Tannin Content (%) | Tannin Amount (g) | Extraction Yield (%) | Tannin Content (%) | Tannin Amount (g) |
| 30°C | 15.90 | 53.85 | 8.56 | 22.12 | 60.92 | 13.48 | 22.60 | 63.96 | 14.47 |
| 50°C | 29.10 | 62.40 | 18.16 | 33.40 | 64.87 | 21.67 | 35.83 | 68.28 | 24.48 |
| 70°C | 33.93 | 68.68 | 23.30 | 41.66 | 71.66 | 32.22 | 43.21 | 72.04 | 31.12 |
| 90°C | 48.53 | 70.97 | 34.44 | 52.73 | 70.86 | 37.36 | 53.55 | 70.45 | 37.72 |
| **Ultrasonic Power** | **Extraction Yield (%)** | **Tannin Content (%)** | **Tannin Amount (g)** | **Extraction Yield (%)** | **Tannin Content (%)** | **Tannin Amount (g)** | **Extraction Yield (%)** | **Tannin Content (%)** | **Tannin Amount (g)** |
| 40W | 33.21 | 65.10 | 21.61 | 40.50 | 65.79 | 26.66 | 43.89 | 69.00 | 30.28 |
| 60W | 43.16 | 66.91 | 28.87 | 46.45 | 68.48 | 31.82 | 48.58 | 69.09 | 33.57 |
| 80W | 46.01 | 65.95 | 30.36 | 51.38 | 68.17 | 35.02 | 54.76 | 67.03 | 37.41 |
| 100W | 58.58 | 67.47 | 39.52 | 60.37 | 66.79 | 40.31 | 62.83 | 64.05 | 40.24 |

## TABLE IX
## Comparison of ultrasonic and conventional extraction yields at constant temperatures.

| | Extraction Temperature | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 30°C | | | 50°C | | | 70°C | | |
| Extraction Method | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h |
| Conventional | 15.93 | 22.10 | 22.60 | 29.10 | 33.4 | 35.80 | 33.98 | 41.68 | 43.21 |
| Ultrasound 40W | 14.31 | 16.74 | 20.52 | 28.71 | 33.20 | 36.83 | 36.83 | 41.61 | 44.22 |
| Ultrasound 60W | 14.58 | 17.55 | 21.87 | 29.32 | 34.07 | 37.70 | 38.28 | 42.77 | 45.24 |
| Ultrasound 80W | 15.66 | 19.98 | 23.76 | 33.49 | 39.65 | 43.79 | 39.15 | 43.21 | 45.96 |
| Ultrasound 100W | 20.38 | 23.22 | 27.67 | 35.27 | 40.45 | 44.80 | 41.90 | 44.66 | 46.69 |

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## REFERENCES


1. Haslam, E., 1989, Chemistry&Pharmacology of Naturel Products: Plant Polyphenols Vegetable Tannins Revisited, Cambridge University Press, ISBN 0 521 32189 1, pp. 2.
2. Naczk, M and Shahidi, F.; Extraction and Analysis of Phenolics in Food. Journal of Chromatography A **1054**, 95-111, 2004.
3. Sivakumar, V., Anna, J.L., Vijayeeswarri, J and Swaminathan, G.; Ultrasound Assisted Enhancement In Natural Dye Extraction From Beetroot For Industrial Applications And Natural Dyeing Of Leather. *Ultrasonic Sonochemistry* **16**, 782-789, 2009.
4. Palma, M., Piňeiro, Z and Barroso, C.G.; In-Line Pressurized-Fluid Extraction – Solid-Phase Extraction for Determining Phenolic Compounds in Grapes. *Journal of Chromatography A* **968**, 1-6, 2002.
5. Llompert, M.P., Lorenzo, R.A., Cela, R., Li, K., Bálanger, J.M.R and Paré, J.R.C.; Evaluation of Supercritical Fluid Extraction, Microwave-assisted Extraction and Sonication in the Determination of Some Phenolic Compounds from Various Soil Matrices. *Journal of Chromatography A* **774**, 243-251, 1997
6. Herrera, M.C and Luque de Castro, M.D.; Ultrasound-assisted Extraction of Phenolic Compounds from Strawberries Prior to Liquid Chromotographic Seperation and Photodiode Array Ultraviolet Detection, *Journal of Choromatography A* **1100**, 1-7, 2005.
7. Hromádková, Z., Ebringerová, A and Valachovic, P.; Ultrasound-assisted Extraction of Water-Soluble Polysaccharides from the Roots of Valerian (*Valeriana officinalis* L.). *Ultrasonics Sonochemistry* **9**, 37-42, 2002.
8. Sivakumar, V., Ravi Verma, V., Rao, P.G and Swaminathan, G.; Studies on the Use of Power Ultrasound in Solid-Liquid Myrobalan Extraction Process. *Journal of Cleaner Production* **15**, 1813-1818, 2007.
9. Sivakumar, V., Jayapriya, J., Srinandini, P and Swaminathan, G.; Ultrasound Assisted Enhancement in Wattle Bark (Acacia Mollissima) Vegetable Tanning Extraction for Leather Processing. *JALCA* **104**, 375-383, 2009.
10. Rodrigues, S and Pinto, G.A.S.; Ultrasound Extraction of Phenolic Compounds from Coconut (Cocos nucifera) Shell Powder. *Journal of Food Engineering* **80**, 869-872, 2007.
11. Markom, M., Hasan, M., Daud, W.R.W., Singh, H and Md Jahim, J.; Extraction of Hydrolysable Tannins from Phyllanthus niruri Linn.: Effects of solvents and Extraction Methods. *Separation and Purification Technology* **52**, 487-496, 2007.
12. Herrera, M.C and Luque de Castro, M.D.; Ultrasound-assisted Extraction for the Analysis of Phenolic Compounds in Strawberries. *Anal. Bioanal. Chem.* **379**, 1106-1112, 2004.
13. Morera, J.M., Bartoli, E., Bacardit, A., Olle, L., Diaz, V and Shendryk, A.; Effect of Ultrasound on Watery Dissolution of Vegetable Extracts Used in Leather Tanning. *JALCA* 103, 151-157, 2008.
14. Mason, T.J.; The Uses of Ultrasound in Chemistry. Royal Society of Chemistry Publication, Chapter 1, pp. 1-3, 1990.
15. Lee, K.W and Kim, J.P.; Effect of Ultrasound on Disperse Dye Particle Size. *Textile Research Journal* **71**, 395-398, 2001.
16. Patist, A and Bates, D.; Ultrasonic Innovations in the Food Industry: From the Laboratory to Commercial Production. *Innovative Food Science and Emerging Technologies* **9**, 147-154, 2008.
17. Mason T.J and Zhao, Y.; Enhanced extraction of tea solids using ultrasound. *Ultrasonics* **32**, 375-377, 1994.
18. Schneider, F.H., Rutte, U and Khoo, D.; Investigation of Lipid Release from Rapeseed. *Fette Seifen Anstrichmittel* **87**, 66-74, 1985.
19. Contamine, F., Faid, F., Wilhelm, A.M., Berlan, J and Delmas, H.; Chemical Reactions under Ultrasound. *Chem. Eng. Sci.* **49**, 5865-5873, 1994.
20. Suslick, K.S and Cassadonte, D.J.; Heterogeneous Sonocatalysis with Nickel Powder. *J. Am. Chem. Soc.* **109**, 3459-3461, 1987.
21. Lorimer, *J.P and Mason, T.J.; Sonochemistry Part 1. The Physical Aspects, Chemical* Society Reviews **16**, 239-274, 1987.
22. Capelo, J.L., Filgueiras, A.V., Lavilla, I and Bendicho, C.; Solid-Liquid Extraction of Copper from Slurried Samples Using High Intensity Probe Sonication for Electrothermal Atomic Absorption Spectrometry. *Talanta* **50**, 905-911, 1999.
23. Luque-Garcia, J.L and Castro, M.D.; Ultrasound: A Powerful Tool for Leaching. *Trends in Analytical Chemistry* **22**, 41–47, 2003.
24. Haizhou, L., Pordesimo, L and Weiss, J.; High Intensity Ultrasound-assisted Extraction of Oil from Soybeans. *Food Research International* **37**, 731-738, 2004.
25. Official Methods of Analysis, SLC-113 Determination of Moisture, Society of Leather Technologists and Chemists. 1996.
26. Official Methods of Analysis, SLC-114 Determination of Total Solids, Society of Leather Technologists and Chemists. 1996.
27. Official Methods of Analysis, SLC-115 Determination of Total Solubles, Society of Leather Technologists and Chemists. 1996.
28. Official Methods of Analysis, SLC-116 Determination of Non-tannin Constituents, Society of Leather Technologists and Chemists. 1996.
29. Official Methods of Analysis, SLC-117 Determination of Tannin Matter Absorbable by Hide Powder, Society of Leather Technologists and Chemists. 1996.
30. Mason, T.J., Paniwnyk, L and Lorimer J.P.; The Uses of Ultrasound in Food Technology. *Ultrasonics Sonochemistry* **3**, 253-260, 1996.