Ultrasound Extraction of Valonea Tannin Part II: Effects on Tannin Structure and Tanning Ability

by

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Abstract

Ultrasound extraction of valonea tannin and its effects on extraction yields in comparison with conventional extraction method was examined in our previous study. Since the extraction conditions are very effective on the characteristics of the tannins, in the present part of the study the tannins obtained by conventional and ultrasonic extraction methods were examined comperatively in terms of their tanning ability, filling properties and molecular structure (by MALDI-TOF). The results demonstrated that while increasing extraction temperatures were increasing filling coefficients of the tannins in conventional extraction, increasing ultrasonic power were decreasing the filling coefficients in ultrasonic extraction. The MALDI-TOF analysis results revealed that whereas in conventional extraction, high extraction temperatures (90ºC) caused an onset of some minor fragmentations; in ultrasonic extractions, high ultrasonic powers (100W) caused significant decompositions on valonea tannin.

Resumen

Extracción por ultrasonido de tanino de valonea y los efectos sobre los rendimientos comparados con la extracción por métodos convencionales fueron examinados en un informe previo. Como las condiciones de extracción fueron muy efectivas [en determinar] las características de los taninos, en la presente fase de este estudio, los taninos obtenidos por métodos de extracción convencionales y ultrasónicos, fueron examinados comparativamente en términos de su potencial en curtir, en capacidad de relleno, y estructura molecular (por medio de MALDI-TOF). Los resultados demostraron que mientras en extracciones convencionales los incrementos en temperatura del proceso conllevaron a mejor coeficientes en las propiedades de relleno, con el uso de ultrasonido e incrementadas temperaturas de extracción, resultaron en inferiores coeficientes de relleno. Los resultados del análisis MALDI-TOF indicaron que en el caso de la extracción convencional, altas temperaturas de extracción (90 ºC) causaron una incipiente fragmentación del extracto obtenido; en el caso de extracciones por ultrasonido a altas potencias (100W) se ocasionó una significante descomposición del extracto de valonea.

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INTRODUCTION

As is well known, 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. 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. For this reason there is a growing demand for developing suitable extraction techniques for more efficient and effective extraction of available active matters from the plant materials.[1] Hence in the light of the above concerns, in our previous study we investigated and compared valonea extraction yields by ultrasound and conventional methods.

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] Besides the extraction yields, extraction conditions are very effective on the characteristics of the tannins (especially the hydrolysable ones due to their poly ester structure). For this reason in the present part of our study we tried to investigate how the extraction methods/conditions (ultrasound and conventional methods) did affect molecular structure of the extracted valonea tannin. In order to investigate the changes in valonea structure (possible breakdowns and split offs) molecular weight distribution of the extracted tannins were detected by MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) technique. Additionally the selected extracts obtained by conventional and ultrasonic extraction methods were examined comparatively in terms of their tanning ability and filling properties.

MATERIALS AND METHODS

Material

In order to determine the effects of the tannins extracted by conventional and ultrasonic methods and of different extraction conditions of each method on tanning properties; extracts obtained at the end of the periods that provided the highest yields for each temperature value (30°C-8h, 50°C-8h, 70°C-8h, 90°C-8h) in the conventional method and for each ultrasonic power (40W-8h, 60W-8h, 80W-8h, 100W-8h, 100W-6h) in the ultrasonic method were used in the tanning process of pickled sheep skins. The croupon areas of the pickled sheep skins were used for the tanning trials.

Methods

Tanning of Skins With Extract Solutions

Since total solid amounts of solutions obtained at the end of each extraction process and the percentage of tannin in these solutions showed variances, and tanning process should be carried out with the same amount of active tanning matter in order to compare their tanning effects, while calculating the extract amount to be used over skin weight, both the extract volume and the tannin content of each extract was taken into consideration. After calculating the volumes of solutions obtained at the end of the extraction process, and their total solid substance and tannin contents, the skins were tanned with the needed amount of extraction solutions, to be containing 13% active tanning matter, according to Table 1.

Determination of Tanning Properties of Extracted Tannins

In order to investigate filling properties of the tannins, the thicknesses of the de-pickled pelts (T_P) and the tanned leathers (T_L) were determined by using a thickness gauge with 100 g pressure in wet form. The filling coefficients of the tannins were defined as $(T_L\text{-}T_P)/T_P$.[3] Shrinkage temperatures of the tanned leathers were determined according to IUP 16.[4]

MALDI-TOF Analysis of Extracted Tannins

As well as conventional and ultrasonic extraction methods were investigated for their yields and tanning properties, the possible effects on molecular structure were also tried to be investigated. To this end, re-extractions were performed on the conventional and ultrasonic extraction parameters selected for the tanning processes (for conventional method; 30°C-8h, 50°C-8h, 70°C-8h, 90°C-8h and for ultrasonic method 40W-8h, 60W-8h, 80W-8h, 100W-8h, 100W-6h), and the obtained extracts were dried by using LAB PLANT SD-Basic spray dryer and the molecular weight distributions of obtained powder extracts were examined by MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight). In this way, we tried to establish whether molecular structure was affected by the two extraction methods and different extraction conditions used by these methods.

The samples were dissolved in acetone (4 mg/ml). Then the sample solutions were mixed with an acetone solution (10 mg/ml) of the matrix, for which 2,5 dihydroxy benzoic acid was used. The solutions of the samples and the matrix were mixed in equal amounts and 0.5 to 1 µl of the resulting solution was placed on the MALDI target. After evaporation of the solvent, the MALDI target was introduced into the spectrometer. The spectra were recorded on an Applied Biosystems Voyager-DE STR instrument. The irradiation source was a pulsed nitrogen laser with a wavelength of 337nm.The length of one laser pulse was 3 ns. The measurements were carried out using the following conditions: positive polarity; linear flight path; high mass (20 kV acceleration voltage); 100-150 pulses per spectrum. The delayed extraction technique was used, applying delay times of 200-800ns.[5,6]

TABLE I
Recipe for tanning.

PROCESS	AMOUNT (%)	PRODUCT	TIME
Depickle	300	Water 6 °Be	5 min.
	1.5	HCOONa	30 min.
	X	$NaHCO_3$	120 min.
			pH 4.5-5
Washing	200	Water	10 min.
Tanning	2	Naphthalene phenol sulphone based syntan	20 min.
	X	Extract solution	30 min.
	1	Synthetic fatliquor	
	0.5	Sulphochlorinated synthetic paraffin and combination of emulsifiers	15 min.
	X	Extract solution	30 min.
	1	Synthetic fatliquor	
	0.5	Sulphochlorinated synthetic paraffin and combination of emulsifiers	15 min.
	X	Extract solution	30 min.
	1	Synthetic fatliquor	
	0.5	Sulphochlorinated synthetic paraffin and combination of emulsifiers	5 hours
	X	HCOOH	1 hour
			pH 3.5
Washing	200	Water	10 min.

RESULTS AND DISCUSSION

Findings Regarding Tanning Properties of the Extracted Tannins

Table 2 presents the filling coefficients and shrinkage temperatures of the leathers gained by the tanning process of extracts obtained at the end of conventional and ultrasonic extraction trials which were conducted at different extraction conditions. From the evaluation of the findings given in Table 2, the increase in extraction temperature in the conventional method (30°C – 90°C) resulted in a parallel increase in the fullness properties (42.22% - 51.71%) of the leathers tanned by the obtained tannins. This led us to consider that tannin molecules with higher dimensions passed into the solutions in extractions performed in higher temperatures. Considering of the results of the ultrasonic method, it was observed that the ultrasonic power between 40 W – 80 W used in extractions resulted in a certain reduction in the fullness properties of the leathers provided by the tannins; whereas a considerable reduction was detected in the filling coefficient of the tannin solutions obtained through 6- and 8-hour extractions

especially at 100 W. This finding suggests an occurrence of fragmentation in the chemical structure of the tannins obtained especially by the extraction at 100 W.

Notwithstanding the fact that the rise in the fullness of leathers increased with increasing temperature in the conventional method and decreased with the increase in ultrasonic power in the ultrasonic extraction method; the filling coefficients achieved by the tannins obtained by ultrasonic method at 40, 60 and 80W were higher than the ones achieved by conventional method. While the highest filling coefficient achieved by the tannins obtained from conventional method (90°C, 8 hours) was 51.71%, 60.07 – 53.73 – 52.00% filling coefficient values were attained by ultrasonic method respectively at 40 – 60 – 80 W.

Regarding the shrinkage temperatures (Ts) of leathers obtained by tanning process, shrinkage temperatures observed by all extracts were similar (65.0 – 67.5°C), except for the temperature (61°C) obtained by the extraction process performed at 30°C by the conventional method.

MALDI-TOF Analysis Results

MALDI spectrums of the tannins obtained by conventional method (at 30, 50, 70 and 90°C) are presented in Figures 1 – 4.

TABLE II
Filling coefficient and shrinkage temperature of the leathers tanned with the tannins obtained by conventional and ultrasonic methods

Extraction method	Extraction condition	Filling coefficient (%)	Shrinkage temperature (Ts)
CONVENTIONAL EXTRACTION	30°C, 8 h	42.22%	61.5°C
	50°C, 8 h	45.09%	65.5°C
	70°C, 8 h	45.67%	66.5°C
	90°C, 8 h	51.71%	65.0°C
ULTRASONIC EXTRACTION	40 W, 8 h	60.07%	66.0°C
	60 W, 8 h	53.73%	67.5°C
	80 W, 8 h	52.00%	65.5°C
	100 W, 8 h	41.17%	65.0°C
	100 W, 6 h	31.57%	66.5°C

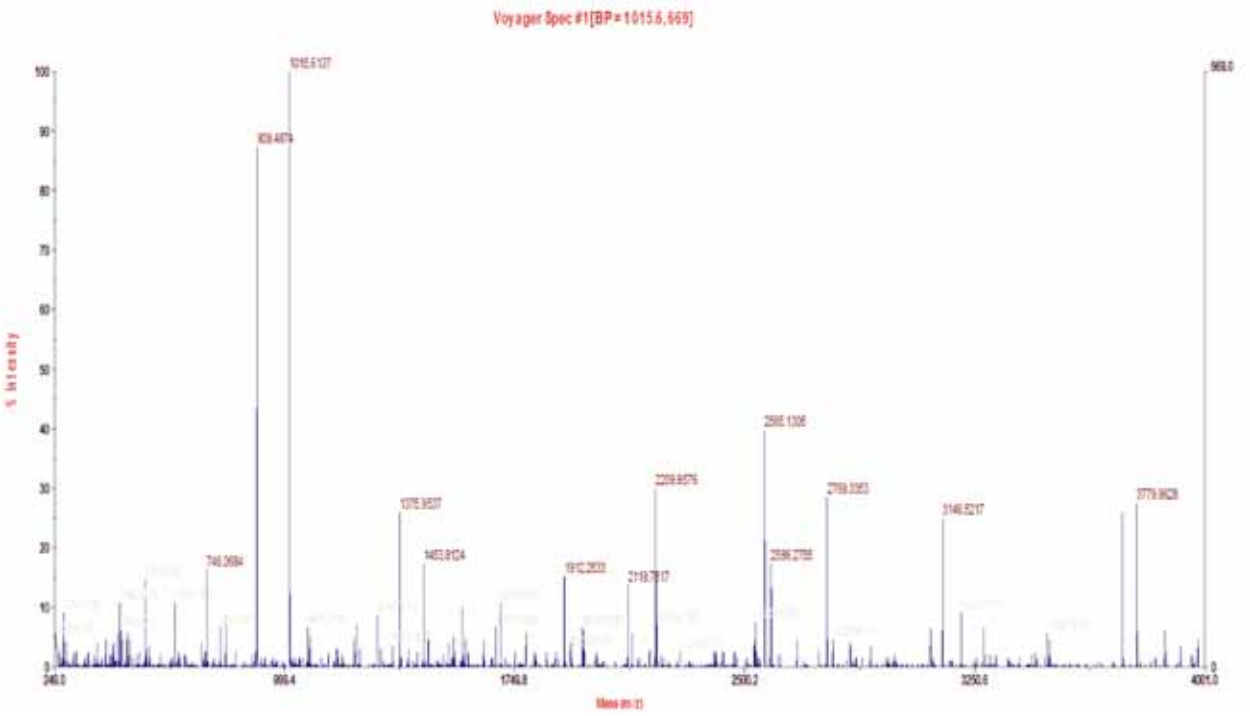

Figure 1. The spectrum of the tannin obtained by conventional method at 30°C-8h.

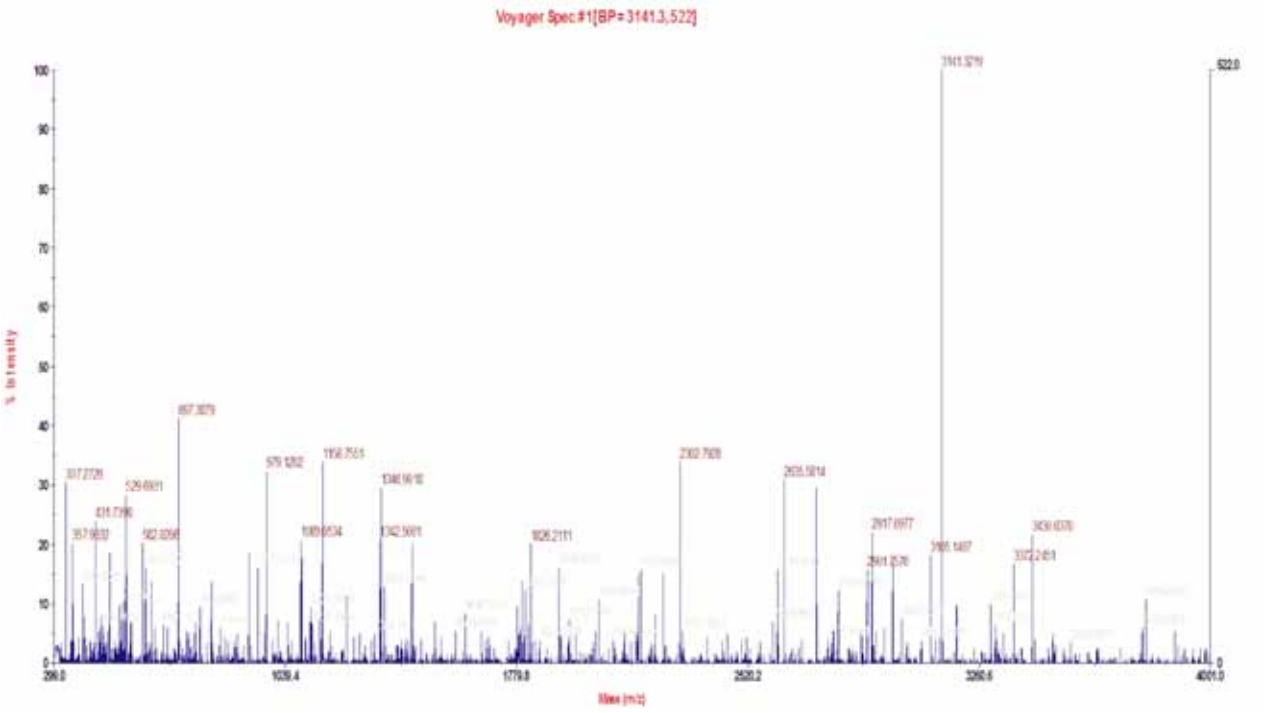

Figure 2. The spectrum of the tannin obtained by conventional method at 50°C-8h.

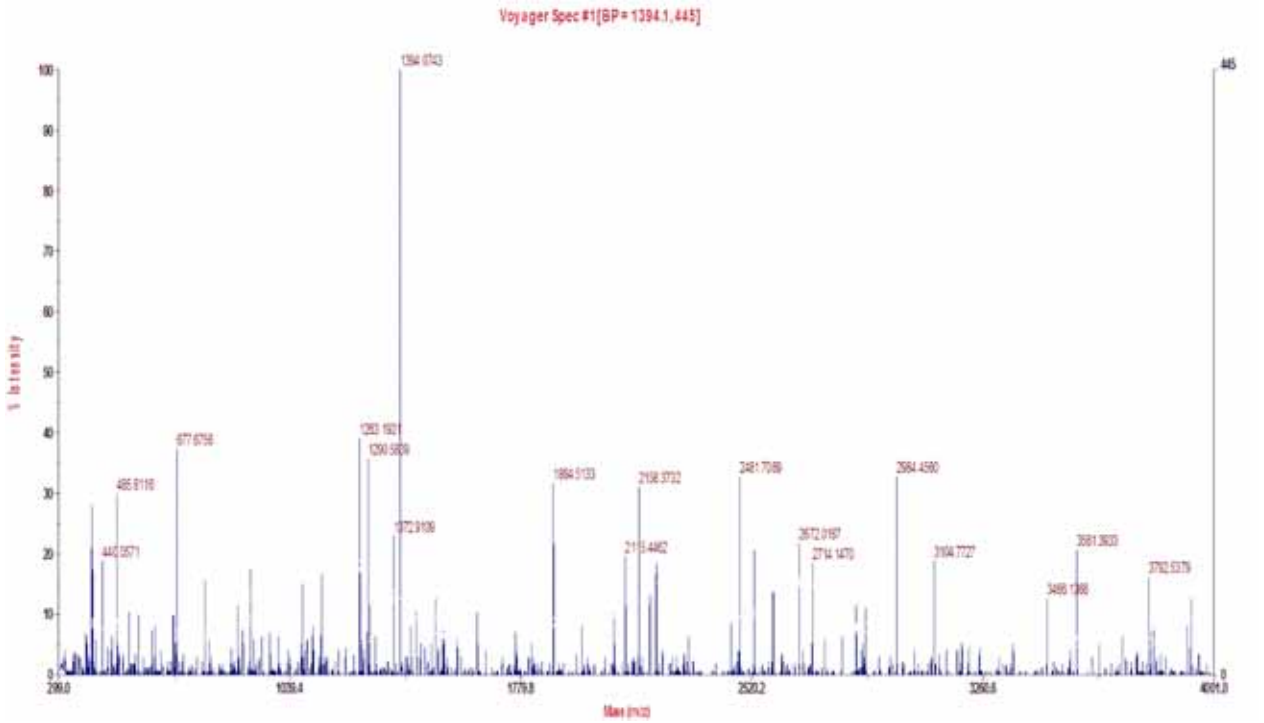

Figure 3. The spectrum of the tannin obtained by conventional method at 70°C-8h.

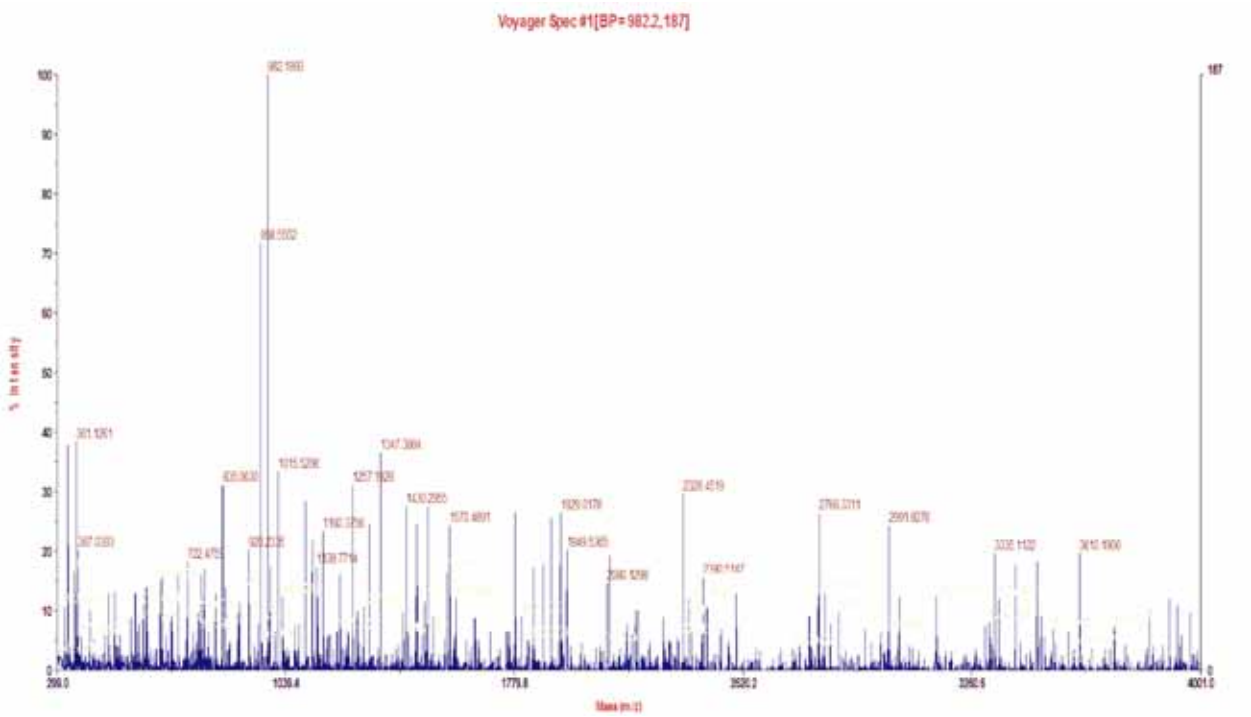

Figure 4. The spectrum of the tannin obtained by conventional method at 90°C-8h.

Considering the MALDI-TOF analysis results (spectra) of the tannins obtained by conventional method, it was observed that the number and intensities of the peaks of higher molecular structures increased with increasing temperature from 30°C to 50°C and 70°C. Which means that greater molecular structures passed into the solutions as a result of the extractions at these temperatures. From the evaluation of the spectra of the tannin obtained at 90°C, although the increased temperature did not cause any significant decomposition in the chemical structure, it was seen that the intensities of some higher molecular structure peaks observed at 50 and 70°C tannin spectrums were decreased, suggesting an onset of some minor fragmentation in the chemical structure. Consequently, although increasing extraction temperatures (up to 70°C) resulted an ease in extraction of higher molecular structures from the raw material (acorn cups), higher extraction temperatures (90°C and/or over) may cause breakdowns and split offs in valoneas structure due to its poly ester structure in conventional method.

MALDI spectrums of the tannins obtained by ultrasonic method (at 40, 60, 80 and 100W) are presented in Figures 5 – 9. From the comparison of the spectrums; there was a partial increase in the number and intensity of peaks that belong to higher molecular structures with increasing ultrasonic power from 40W to 80W. However, a decrease was observed in the area of higher molecular structures, in the spectra of tannins obtained from 6 and 8-hour extractions at 100W. Accordingly, ultrasonic extraction at 100W was thought to have caused fragmentation in tannin structure, due to the high ultrasonic power and the high ambient (extract solution) temperature which was approached to boiling point. The effect of ultrasonic power on ambient temperature was pointed out in our previous study as "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".[7] Consequently, although increasing ultrasonic power (from 40W to 80W) contributed to extraction of higher molecular structures from the raw material (acorn cups), higher ultrasonic powers (100W and/or over) may cause serious breakdowns and split offs in valoneas structure due to high ultrasonic power and significant increase in ambient (extract solution) temperature. As a matter of fact, the filling coefficient value obtained from the tanning process by using the tannins obtained from ultrasound extraction (6 and 8 hours) at 100 W were found to be considerable lower than the filling coefficient results of the other ultrasonic extractions (at 40, 60 and 80W) (Table 2). This result was also associated with the possible negative effect of high levels of non-tannin substances that passed into the 100W ultrasonic extraction on the tanning process.

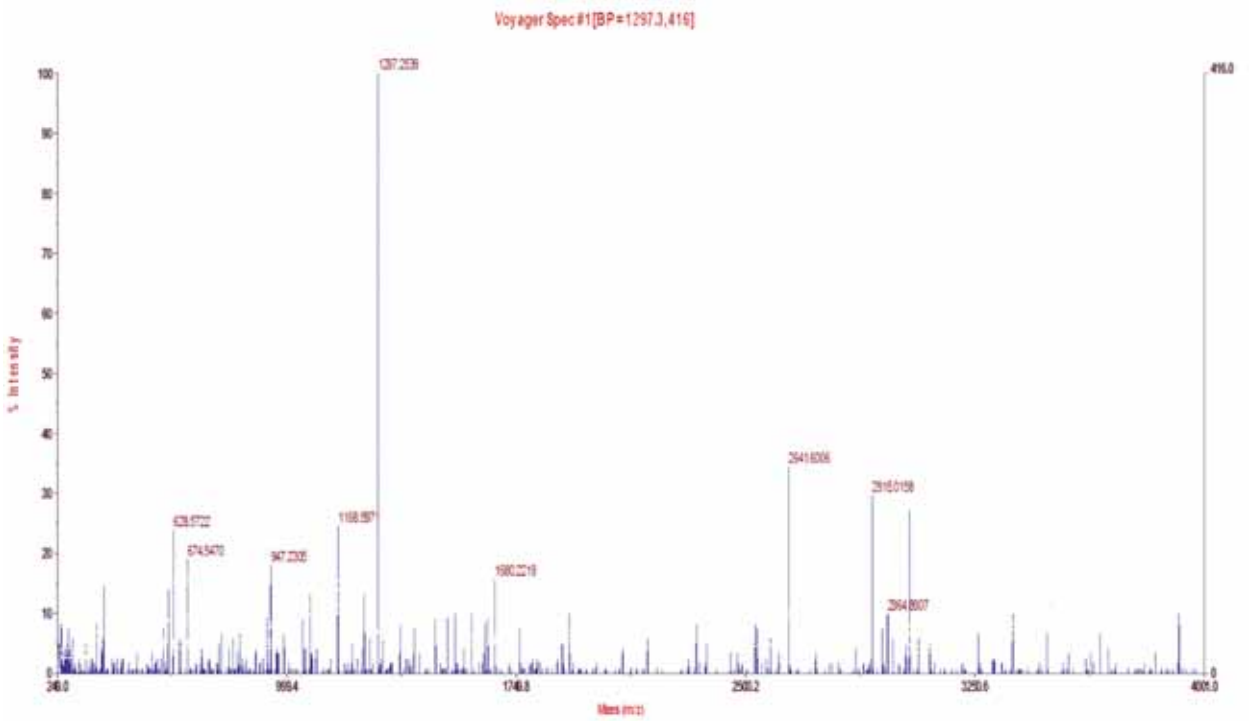

Figure 5. The spectrum of the tannin obtained by ultrasonic method with 40W-8h.

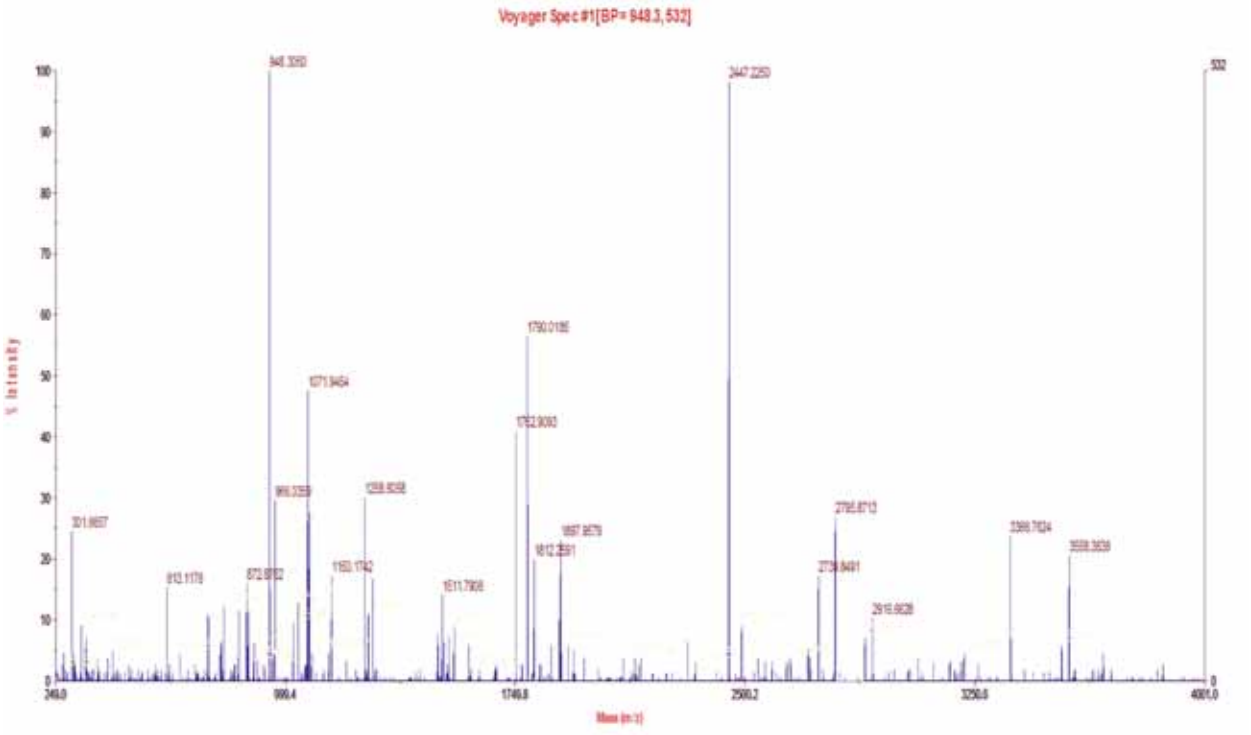

Figure 6. The spectrum of the tannin obtained by ultrasonic method with 60W-8h.

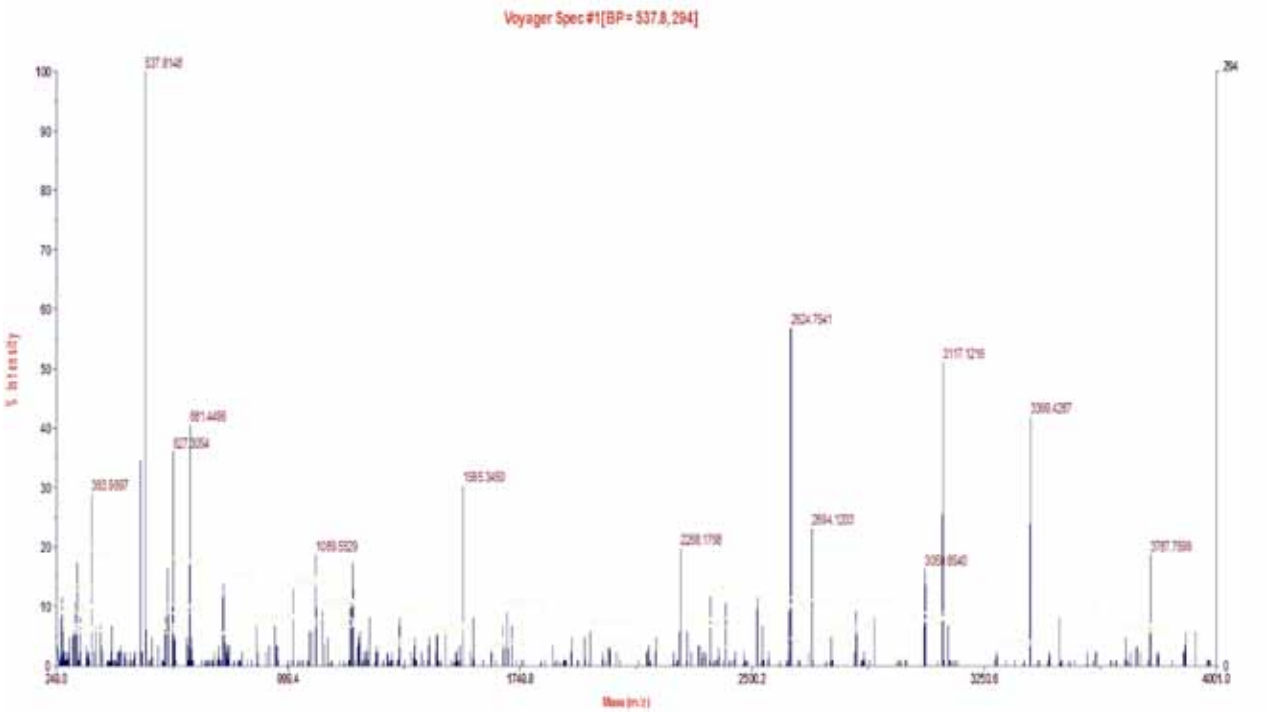

Figure 7. The spectrum of the tannin obtained by ultrasonic method with 80W-8h.

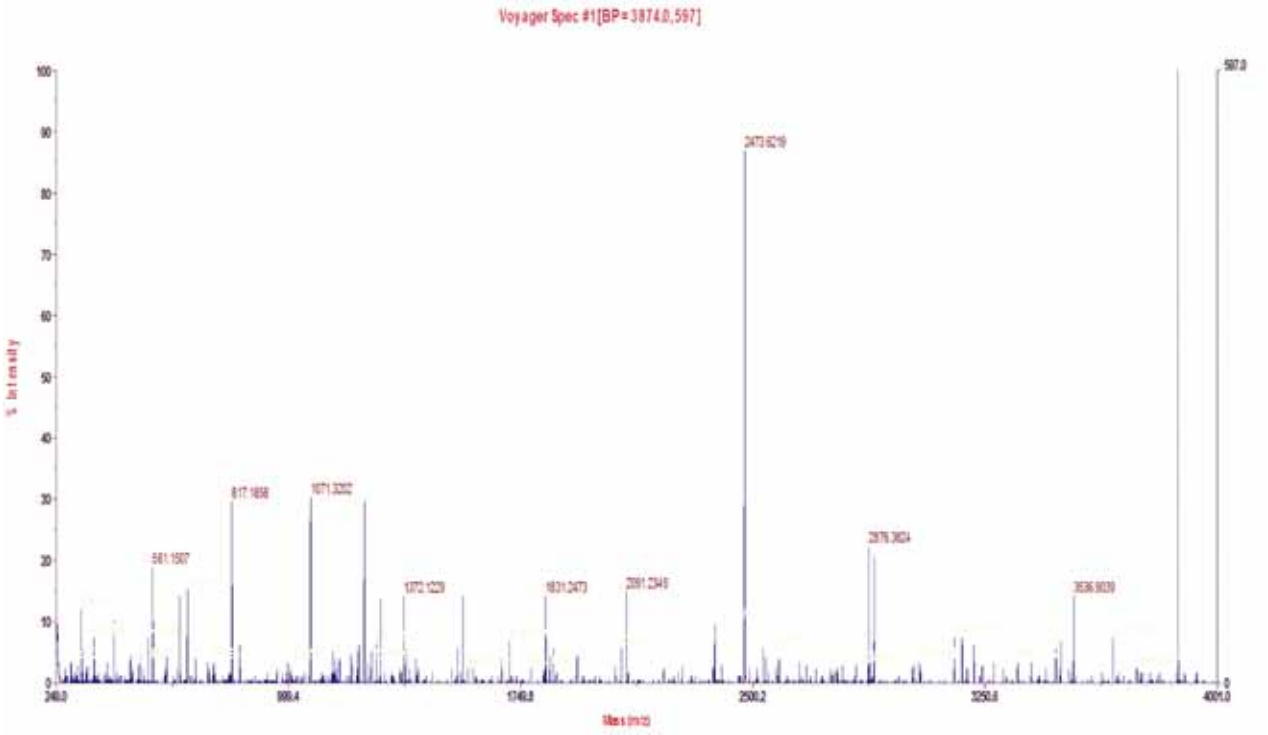

Figure 8. The spectrum of the tannin obtained by ultrasonic method with 100W-6h.

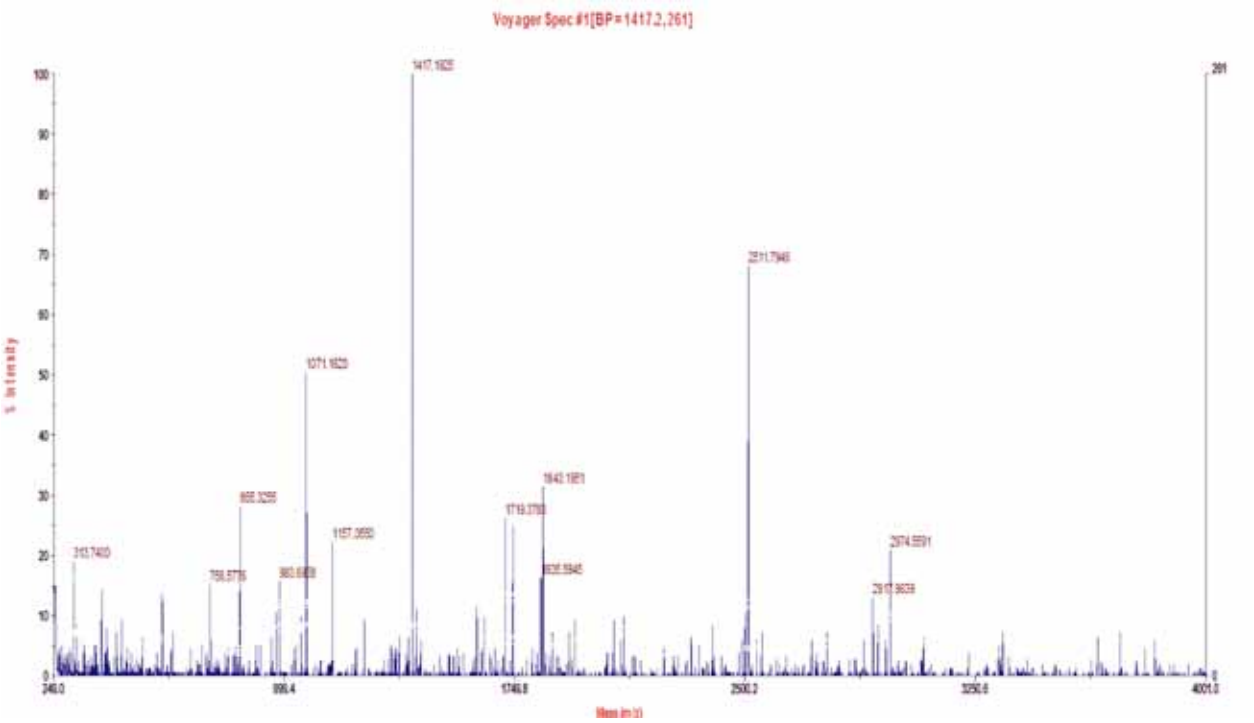

Figure 9. The spectrum of the tannin obtained by ultrasonic method with 100W-8h.

CONCLUSIONS

In the present part of the study the tannins obtained by conventional and ultrasonic extraction methods were examined comperatively in terms of their tanning ability, filling properties and molecular structure (by MALDI-TOF). From the tanning trials it was concluded that all the tannin products, which were obtained at the end of the periods that provided the highest yields for both methods, were used successfully in tanning process. The shrinkage temperatures of the leathers were generally similar (65.0 – 67.5°C) but the filling properties were varying depending on the extraction conditions and methods. Increasing extraction temperatures were yielded tannins with increasing filling coefficients in conventional extraction, which led us to consider that tannin molecules with higher dimensions passed into the solutions in extractions which performed in higher temperatures. In ultrasonic extraction, increasing ultrasonic power was yielded tannins with decreasing filling coefficients, especially at 100 W. This finding suggested an occurrence of fragmentation in the chemical structure of the tannins obtained especially by the extraction at 100 W. The MALDI-TOF analysis results additionally revealed that: In conventional extraction high extraction temperatures (90°C) caused an onset of some minor fragmentation in the chemical structure of the valonea tannin. On the other hand, in ultrasonic extractions at high ultrasonic powers (100W) caused significant decompositions, due to fragmentations and split-offs in structure, on valonea tannin.

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