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Research Article

Determination of Quality in Homemade Vinegars by Spectroscopy and Rheology Methods

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ABSTRACT

This study focused on the determination of quality characteristics of homemade organic vinegars (apple, red hawthorn and yellow hawthorn) which were produced with double fermentation (ethyl alcohol and acetic acid fermentation) method by spectroscopy (UV/Fourier transform infrared) and rheology technique. These absorbance peak values are associated with organic acids and phenolic compounds were determined as an important parameter in the quality evaluation of vinegars. It was determined that the flow curves of all vinegars are compatible with the non-Newtonian flow, which is the behaviour of thickening (dilatant) fluids. From the obtained results, it was thought that the antioxidant and anti-bacterial effect of yellow hawthorn vinegar would be higher due to its higher acetic acid and phenolic compound content compared to the others.

Keywords: *Vinegar, UV spectra, FTIR spectra, Acetic acid, Organic acids, Phenolic compounds, Rheology, Non-newtonian flow, Dilatant fluids*

Ev Yapımı Sirkelerde Kalitenin Spektroskopi ve Reoloji Yöntemleriyle Belirlenmesi

ÖZ

Bu çalışma, çift mayalanma (etil alkol ve akabinde asetik asit mayalanması) yöntemi ile üretilen ev yapımı organik sirkelerin (elma, kırmızı alıç ve sarı alıç) kalitesinin spektroskopi (UV/Fourier dönüşümlü kızılötesi) ve reoloji tekniği ile belirlenmesine odaklanmıştır. Organik asitler ve fenolik bileşiklerle ilişkili bu absorbans tepe değerleri, sirkelerin kalite değerlendirmesinde önemli bir parametre olarak saptandı. Tüm sirkelerin akış eğrilerinin, kıvamlaştırıcı (dilatant) akışkanların davranışı olan Newton olmayan akışla uyumlu olduğu belirlendi. Elde edilen sonuçlardan sarı alıç sirkesinin daha yüksek asetik asit ve fenolik bileşik içeriği nedeniyle antioksidan ve anti bakteriyel etkisinin diğerlerine göre daha yüksek olacağı öngörüldü.

Anahtar Kelimeler: *Sirke, UV spektrum, FTIR spektrum, Asetik asit, Organik asitler, Fenolik bileşikler, Reoloji, Newton olmayan akış, Genişleyen akışkanlar*

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I. INTRODUCTION

Vinegar is basically a combination of acetic acid and water made by a two-stage fermentation process of carbohydrate-rich foods. Traditional vinegars, also known as handmade vinegars are produced by first alcohol fermentation of foods rich in carbohydrates and then acetic acid fermentation [1]-[3]. On the other hand, commercial vinegars are produced by the rapid fermentation process, which is defined as a submerged culture fermentation in which bacterial culture is suspended in fruit liquid before adding air for oxygenate [4]. Until the last century, vinegars were at the forefront with their protective properties in food products, while they have been included in human life with many features that are beneficial to human health recently. Vinegars, which are suitable for consumption for a long time, are very rich in antioxidants, which are a very important substance in preventing oxidative stress, which is a big danger to human health. In particular, fruit vinegars are very good in terms of antioxidants compared to an equivalent fruit juice and are preferred [5]-[10]. The phenolic and organic acid compounds of the vinegars fermented from a wide variety of fruits by different processes (traditional or commercial) are different and accordingly their antioxidant capacity is also at different levels [11], [12].

Vinegars have many functional properties that can provide great benefits for human health due to their phenolic and organic acid compounds [11]-[13]. These fermented products have very protective and even healing properties due to organic acids such as acetic acid in their structure against bacteria and infections that cause illness [14]. In animal experiments, it was determined that vinegars are very effective in the regulating of the blood glucose and lipid levels [15]. On the other hand, it was observed that this miracle food was effective in increasing benign cholesterol values and decreasing malignant cholesterol values [16]. The effect of vinegar on cancer and obesity diseases, which are the two threatening factors of today's human life, has been recently investigated by the scientific community [15], [17]. Studies have shown that in different types of cancer, vinegars have been found to greatly inhibit the proliferation of cancer cells, in other words, have an anti-cancer effect [17]. Furthermore, it was concluded that acetic acid, which is the most basic structure of vinegar, can be effective in obese rats losing their unwanted weight [18]. Another very important benefit of vinegar is on cardiovascular and high blood pressure diseases. Researchers have shown that different vinegars cause very positive effects on these two diseases [19], [20].

In the literature, classification, content and quality analysis of commercial vinegars produced by industrial methods has been the subject of many studies [1], [21]-[24]. In these studies, the ultraviolet spectroscopy (UV) [25], inductively coupled plasma mass spectrometry [26], Fourier transform infrared (FTIR) spectroscopy [22], principal component [27], graphite furnace atomic absorption spectrometry [28], gas chromatography-mass spectrometry (GS-MS) [29] and high performance liquid chromatography (HPLC) [30] analysis techniques were used. In this study for the first time, the quality analyses of apple, red hawthorn and yellow hawthorn vinegars produced at home using traditional method (ethyl alcohol and acetic acid dual fermentation) were performed using Rheology technique, UV and FTIR spectroscopy together. Organic acid contents and flow behaviours that determine the quality of homemade apple, red hawthorn and yellow hawthorn vinegars will be determined using the relevant measurements and then quality comparison will be made with each other. The main purpose of this study is to compare the quality of homemade traditional vinegars, which have become widespread in our society recently, with commercial vinegars produced by periodic and legal controls. Thus, it will be possible to reach a conclusion about whether homemade vinegar can be consumed safely or not.

II. MATERIAL AND METHODS

A. PRODUCTION OF APPLE, RED HAWTHORN AND YELLOW HAWTHORN TRADITIONAL HOMEMADE VINEGARS

Amasya type apples were carefully picked from a tree that did not use any agricultural pesticides or poisons, especially when it was at its sweetest. The red and yellow hawthorn fruits were picked at full maturity time from trees at the same altitude and far away from the pathways of vehicles containing lead in their exhaust fumes. The collected apple and hawthorn fruits were washed in the water with 10% grape vinegar for 4 hours to remove unwanted harmful bacteria from the fruits that would adversely affect a healthy fermentation. After the washing process was completed, the fruits were left to dry for 24 hours in a sterile environment with sunlight to increase the effectiveness of phenolic compounds. In order to increase the surface area thought to speed up the ethyl alcohol fermentation, 1 kilogram (kg) of apple fruits were cut into small pieces and then placed into 2.5 litre (L) glass containers. Then, 1 kg of red and yellow hawthorn fruits were gently crushed and filled in a 2.5 L glass container, including its seeds. The 1 L of sterilized water for each, which was boiled and then cooled in order to kill the harmful microorganism inside, was added separately to these three glass containers half full of fruit. The 0.5 L volume part of glass containers were left empty in order to ensure a healthy oxygen flow, which will provide the second fermentation (acetic acid) of fruits. The prepared fruit and water mixtures were kept in closed glass containers for ethyl alcohol fermentation process in a dark room at 27 °C temperature, without exposure to sunlight for 3 months. This mixture, in which ethyl alcohol fermentation is desired to occur more quickly, was mixed with periods of 24 hours. After completion of the first fermentation, 100 mL of pure homemade grape vinegar was added to each glass container to start the acetic acid fermentation following the completion of the first fermentation. The glass containers to which pure vinegar was added were covered with a thin cloth allowing oxygen flow in order for acetic acid fermentation to take place in a healthy and successful way. All of the ethyl alcohol in the glass containers was converted to acetic acid thanks to the second fermentation lasting 6 months in the room with 27 ˚C temperature. Completing the second fermentation, the apple, red and yellow hawthorn samples were rested for 2 months by closing with lids that do not allow air flow to improve the vinegar aroma and taste. Finally, vinegars produced by homemade traditional methods were prepared for rheology and spectroscopy measurements.

B. SPECTROSCOPY AND RHEOLOGY MEASUREMENTS FOR TRADITIONAL HOMEMADE VINEGARS

The optical properties of apple, red hawthorn and yellow hawthorn vinegar were carried out by UV-spectroscopy technique using A 360 Spectrophotometer device. In order to determine the absorbance values of the vinegars, rectangular quartz cuvettes (3.5 mL volume) with path length of 1 cm were preferred and deionized water was used as the baseline in the measurements. The UV spectra for approximately 3% vinegar (0.1 mL) aqueous solutions were recorded at room temperature (RT) in the wavelength range of 190 nm to 600 nm. The absorbance coefficient values of homemade vinegars were calculated using the absorbance values depending on the wavelength. Energy band gaps of all samples were calculated using Tauc plots obtained from absorbance values.

Flow behaviours (rheological properties) of the homemade vinegars were determined using a Rheometer compatible with the measurement of liquids (Malvern Kinexus Pro.). In this preparation phase, 5 mL of nitric acid, 3 mL of hydrochloric acid, 2 mL of hydrogen peroxide, 50 mL of 1% nitric acid distilled water solution and 3.1 mL of vinegar were prepared severally for all the samples. Shear stress values of the all vinegars were calculated from steady state shear rates starting from a single point and changing step by step. The shear rate for all homemade vinegars was recorded range of 10^{-3} s^{-1} to 10^{2} s^{-1} at RT. After the shear stress and shear rate values were determined, the viscosity values of the vinegars were calculated based on them.

Chemical functional groups, functional group orientations and contents for all samples were investigated using Bruker Vertex 70-FTIR, which allows infrared spectra measurements. FTIR measurements for all the homemade vinegars were carried out 4 cm^{-1} resolution for each spectrum and in the wavenumber range of 4200 cm^{-1} to 400 cm^{-1} with 1 cm/s scanning speed at RT. Also, infrared spectral measurements were repeated three times for each of the homemade vinegars to make certain that the FTIR spectroscopy outputs.

C. THEORY

The Lambert-Beer-Bouguer law is used to most comprehensively describe the UV spectra relating to the nature of an electromagnetic light. The simplest form of the relevant law defining the absorbance of materials is as given in the following equation [31].

$$A = \log(I_0/I) = \varepsilon bc \quad (1)$$

Here, A, I_0, I, ε, b and c are define to the absorbance of material, the intensity of the incident light, the intensity of the transmitted light, the extinction coefficient, the length of path the light passes and dose of the absorbing, respectively. The relationship between absorbance and the transmittance is presented as $A = -logT \; and \; T = (I/I_0)$. In order to see the effect of material geometry on absorption, the absorption coefficient is calculated using the absorbance values by the following equation [32].

$$\alpha = 2{,}303.A/t \quad (2)$$

In this equation, α is the absorption coefficient and t is the thickness of the tube. Tauc plots were obtained by using the absorption coefficient values of the material. The optical energy gaps of the material were calculated using the slope of the Tauc plots, which a function of the photon energy. The relationship between the Tauc plots and the photon energy, which allows the calculation of the forbidden energy gaps, is as in the following equation [33]-[36].

$$\alpha h\nu = B(h\nu - E_g)^n \quad (3)$$

Where, h is the Planck's constant, ν is the frequency of incident photon, B is a constant that depends on the electron mobility and the transition probability, E_g is the forbidden energy gap and n is the an index that correspond to the nature of the transition.

The flow behaviour of a fluid is related to the shear stress and the applied shear rate perpendicular to the surface of this stress. If there is a direct proportion between these two values that determine the flow behaviour, it indicates that the fluid has Newtonian flow behaviour. In such liquids compatible with Newtonian flow, the viscosity equation is as follows [37], [38].

$$\tau = \eta\dot{\gamma} \quad (4)$$

In this equation, τ, η and $\dot{\gamma}$ are represent the shear stress, the viscosity and the shear rate in succession.

Non-Newtonian flow behaviour of liquids such as dilatant fluids and pseudo-plastic is given by the Power Law model. In this model, the shape, particle size, particle distribution and the interaction of particles of liquids with each other are extremely important. The Power law equation for non-Newtonian fluids is as follows [39], [40].

$$\eta = K\,\dot{\gamma}^{m-1} \quad (5)$$

Here, K and m are depicted the consistency coefficient and the flow behaviour index, respectively. Dilatant flow behaviour occurs when m >1 in the power law.

III. RESULTS AND DISCUSSION

The evolution of UV spectra for 0.1 mL dose of apple, red hawthorn and yellow hawthorn vinegars in the wavelength range of 190 nm to 600 nm is shown in the Figure 1. The variation of the absorbance and absorption coefficient of the vinegars produced at home by traditional methods with the wavelength is presented in the Figures 1a and 1b, respectively.

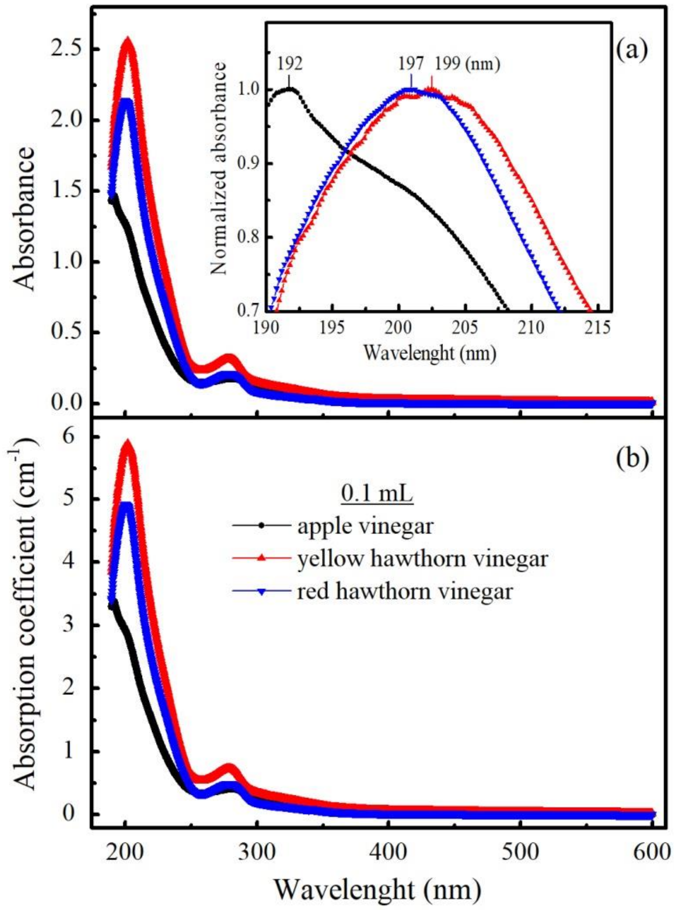

Figure 1. *(a) UV absorbance spectrum and (b) wavelength variation of absorption coefficient (α) for apple, yellow hawthorn and red hawthorn vinegars*

It has been observed that the absorbance values of red and yellow hawthorn vinegars displayed similar behaviour with wavelength, while apple vinegar behaves differently. It was determined that the absorbance of apple, red and yellow hawthorn homemade vinegars had two peaks in the low wavelength region (between 190 nm and 300 nm wavelength). The peak values of the absorbance for the vinegars between 190 nm and 240 nm wavelengths are originated from acetic acid, which is the main source of antibacterial properties [1], [8], [11]. The difference in the absorbance peak values in this wavelength region is related to the acetic acid concentration [41]-[45]. In this case, it was determined that yellow hawthorn vinegar with the highest absorbance peak values had higher acetic acid concentration than the others. Absorbance peaks originated from phenolic compounds, which are the source of the antioxidant properties of vinegars, can be seen between 250 nm and 300 nm wavelength [9], [11], [46]. The peak values of the absorbance spectra of the vinegars show a shift effect with decreasing wavelength due to the bathochromic effect arising from the colour pigments of the fruits and the structure of organic acid molecules [1], [47]. This shift effect is seen more clearly in the normalized absorbance spectrum given in the inset graph in Figure 1a. In the Figure 1b, the wavelength evolution of the absorption coefficients for homemade vinegars was determined to be similar to the absorbance spectra. The absorption coefficient spectra have two peaks, which are reflection of the geometries and optical transitions of molecular of the vinegars. It was predicted that the geometries of the vinegars have little effect on the absorption coefficient peak values between 190 nm to 240 nm and 250 nm to 300 nm wavelength, while the main effect is originated from π-π* the optical transition of molecules of phenolic compound and organic acids [48], [49]. It was concluded that the highest organic acid and phenolic compound concentration belonged to yellow hawthorn vinegar from the absorption coefficient peak values of the samples.

The variation photon energy of the absorption coefficient and graph of $(\alpha h\nu)^2$ versus $(h\nu)$ (Tauc pots) for apple, red and yellow hawthorn vinegars is shown in the Figure 2a and 2b, respectively.

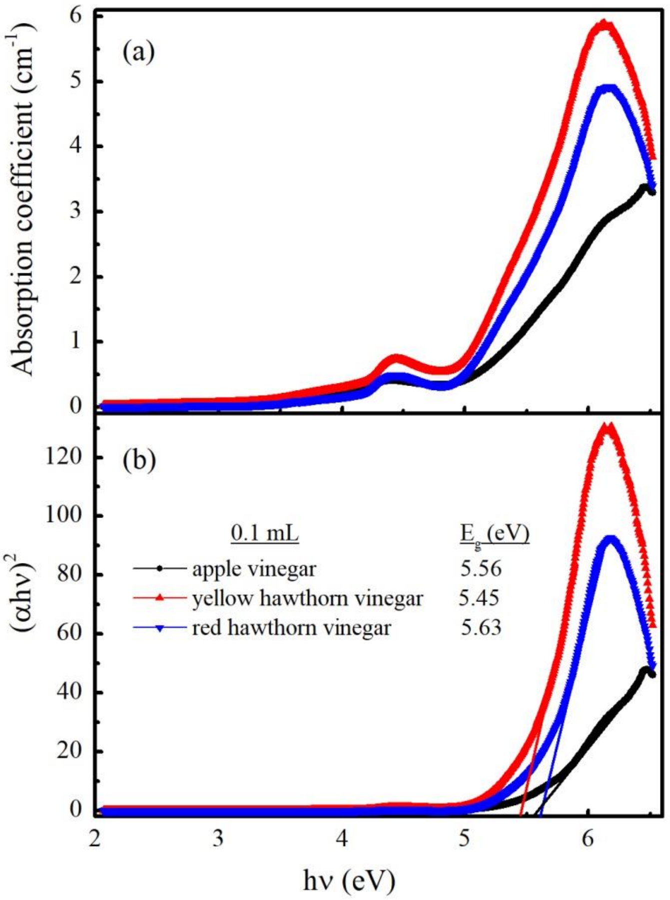

Figure 2. *(a) Energy evolution of absorption coefficient (α) and (b) plots of (αhν)^2 versus hν for apple, yellow hawthorn and red hawthorn vinegars*

It was observed in the Figure 2a that the absorption coefficient values of apple, red and yellow hawthorn vinegars had two peaks depending on the photon energy. It was determined that the absorption coefficient values of red and yellow hawthorn vinegars with energy evolution are very close to each other, while apple vinegar behaved differently. The peak values of the absorption coefficient values for homemade vinegar in the energy region between 4 eV and 5 eV are caused by the phenolic compounds in the structure of the vinegars [1], [12], [32]-[34], [48], [49]. It was observed that the peak values in this region originated from the phenolic compound concentration, which is an indicator of antioxidant effect, were higher in yellow hawthorn vinegar compared to the others [12]. Also, it was thought that the peak values of the absorption coefficient in this energy region were affected by the interaction of phenolic compounds with each other. The peak values of the absorption coefficient of vinegars in the energy region between 5.5 eV and 6.5 eV (high energy region) are due to the presence of acetic acid [1], [11], [32]-[34], [48], [49]. In addition to, the values of the peaks in this region are related to the acetic acid concentration of the vinegar. The shifts in the peaks of the absorption coefficient in this region are the result of the energy gap contraction originated from quantum-confinement on the spectra of acetic acids. [50]. The UV spectra of the apple, red and yellow hawthorn vinegars were relation to the energy band gaps and these optical band gaps were calculated using Tauc's plots shown in the Figure 2b. The absorption coefficient values given in the Figure 2a, which determine whether the electron transitions of the vinegars are direct or indirect, are the main determinants of the samples optical energy gap values [32], [51]. It was observed that Tauc's plots of homemade vinegars were not affected by the increase of energy in the region below 5 eV energy value, on the other hand, they had peak values in the above 5 eV energy region. The optical energy gap values were calculated from the gradient of the $(\alpha h\nu)^2$ versus $(h\nu)$ graphs at the point where the Tauc's plots of the samples reached the peak values. The optical energy gap values of apple, red and yellow hawthorn vinegars were recorded as 5.56, 5.63 and 5.45 eV, respectively. It was determined that the increase in optical energy gaps related to the quantum confinement effect of organic acids and phenolic compounds in the structure of vinegars is compatible with Planck's Rayleigh-Jeans law [32]-[34], [51], [52]. The high optical energy gap value of red hawthorn vinegar compared to the other is related to the shorter release time originated from the lower concentration of acetic acid in its structure.

Shear rate variation of viscosity for apple, yellow hawthorn and red hawthorn vinegars in the shear rate range of 10^{-3} s^{-1} to 10^{2} s^{-1} is depicted in Figure 3.

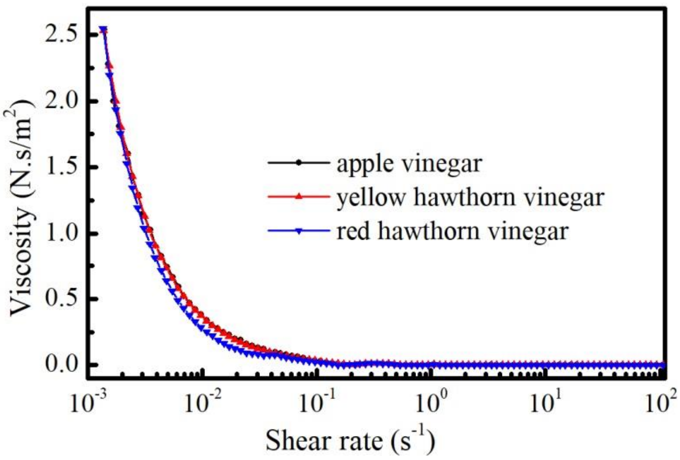

Figure 3. *Shear rate evolution of viscosity for apple, yellow hawthorn and red hawthorn vinegars*

It was determined that the viscosity of apple, red and yellow hawthorn vinegars decreases exponentially with increasing shear rate in the low shear rate region (10^{0} s^{-1}). On the other hand, it was observed that the viscosity behaves independently from the increasing shear rate in the high shear rate region. In the lower shear rate region, the viscosity of all samples has the highest value since the resistance to flow rate is the highest. It was concluded that the molecular size distributions of the vinegars and the interactions of the organic acid molecules are effective at high viscosity values in this region [53]. All vinegars exhibit stable flow behaviour in the high shear rate region. This stable flow behaviour was interpreted as a result of the healthy completion of the fermentation processes of all vinegars. In addition, the similar flow behaviours of the apple, red and yellow hawthorn vinegars were attributed to the healthy production process and their quality. This similar behaviour is presented more clearly in Figure 4 where the flow curve is given. It was found that the flow behaviour of homemade vinegars produced by traditional method is compatible with non-Newtonian dilatant fluid behaviour [39], [40], [54].

Flow curves (shear rate evolution of the shear stress) for the apple, red and yellow hawthorn vinegars in the shear rate of 0 s^{-1} to 300 s^{-1} is presented in Figure 4.

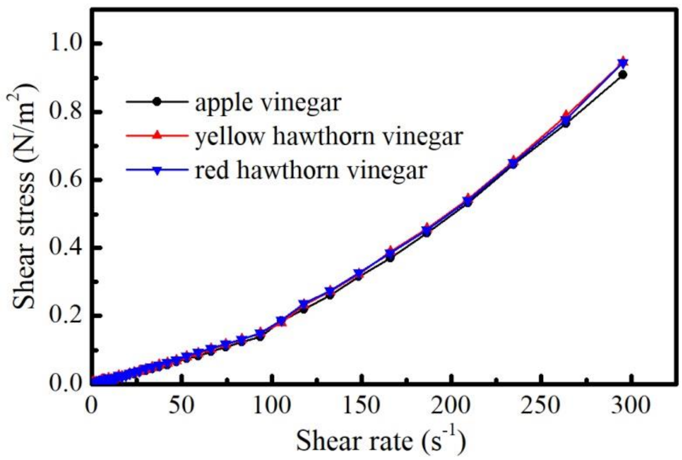

Figure 4. *Flow curves for apple, yellow hawthorn and red hawthorn vinegars*

The shear rate evolution of the shear stress for apple, red and yellow hawthorn vinegars was found to be consistent with the flow behaviour presented in Figure 3. It was determined that the flow curves of these vinegars produced by traditional method were compatible with the flow behaviour of non-Newtonian thickening liquids (dilatant fluid). Dilatant flow (shear thickening behaviour) usually occurs when liquids move from a steady state to an unstable state (flocculation). In this flow behaviour of vinegars, there have an increase in shear viscosity due to the increase in shear stress [54]-[56]. It is observed that the flow behaviour of the samples in the high shear rate region (200 s^{-1} to 300 s^{-1}) was more consistent with the Newtonian flow, so they show stable flow. The main reason for the steady flow behaviour observed in this region was predicted as the decrease in the void volume between the particles with increasing shear rate, decrease in friction and consequently decrease in shear viscosity. Based on this, it was determined that the viscosity of homemade vinegars in the region of high shear rate is not affected by shear stress, so it can start to flow with a small force. As a result, it has been concluded that the flow curves of three vinegars produced at home with the traditional method with dilatant liquids flow are compatible with the Power law model [39], [40], [53], [54].

Fourier transform infrared spectroscopy (wavenumber variation of absorbance) for apple (a), yellow hawthorn (b) and red hawthorn (c) vinegars in the wavenumber range of 4200 cm^{-1} to 400 cm^{-1} are depicted in Fig. 5, in succession.

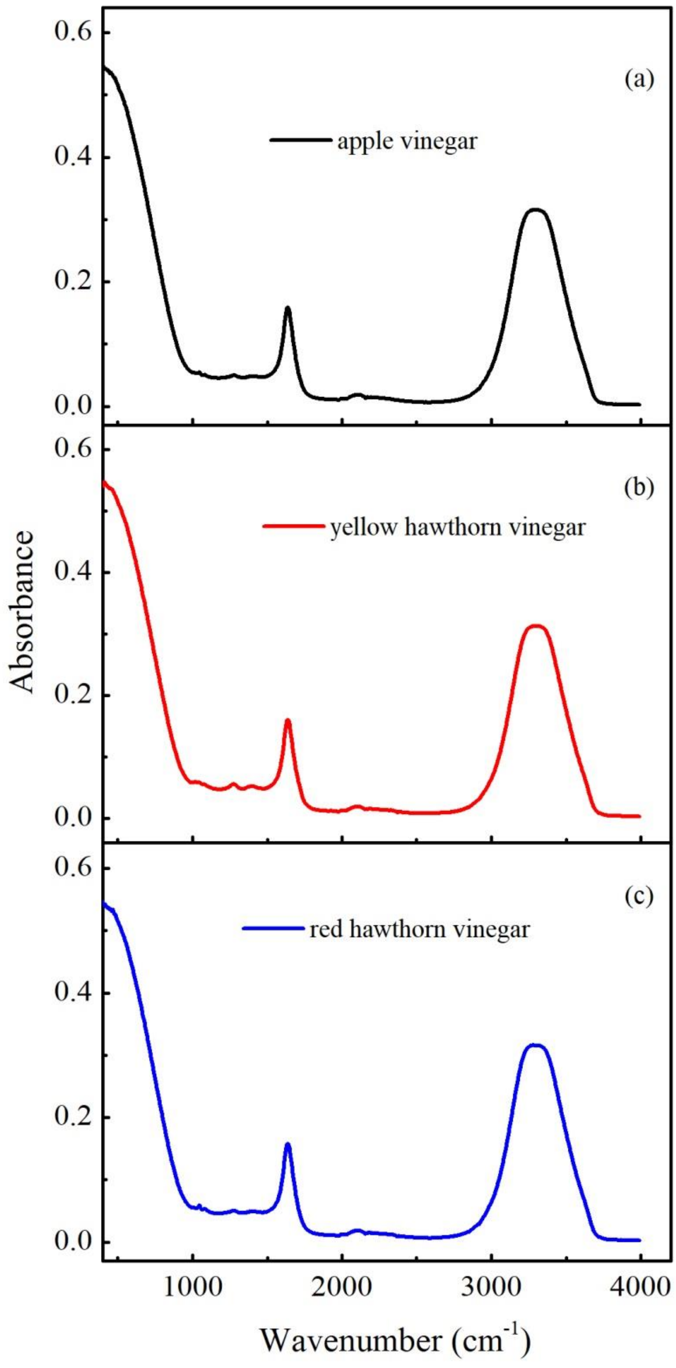

Figure 5. *FTIR spectra of (a) apple, (b) yellow hawthorn and (c) red hawthorn vinegars*

The spectral fingerprints of the FTIR spectra for apple, red and yellow hawthorn vinegars in the middle infrared frequency region are very similar. This similarity in spectral fingerprints were interpreted that the conversion processes of all fruits into vinegar are healthy. It was observed that wavenumber evolution of FTIR spectra for all homemade vinegars has two peaks in the around 3300 cm^{-1} and 1635 cm^{-1} wavenumbers. The peaks of the spectral fingerprints of the vinegars around 3300 cm^{-1} wavenumber are originated from H-O-H asymmetric stretching vibration and water absorption. The C=C stretch and the H-O-H symmetrical stretching vibration are the main reasons, which homemade vinegars have absorption peaks around 1635 cm^{-1} wavenumber. The small differences in these two important peak values of the FTIR spectra of the samples are caused by the different concentrations of the organic acids and phenolic compounds in the structure of the vinegar. Also, it was also thought that the small spectral fingerprints of vinegars around 2098 cm^{-1}, 1403 cm^{-1}, 1271 cm^{-1} and 1043 cm^{-1} wavenumbers are the result of presence of the hydroxyl groups in ethyl alcohol, C-

H, C-O and C-C bonds. It was concluded that the characteristic peaks of spectral fingerprints of the apple, red and yellow hawthorn vinegars by produced traditional method are compatible with the mixture of acetic acid and water that forms the structure of the vinegars [21], [57], [58].

IV. CONCLUSION

In this work, it is aimed to perform the quality analysis of apple, red and yellow hawthorn vinegars produced at home with the traditional method (long process with two fermentation) using the UV, FTIR spectroscopy methods and rheology technique. For this purpose, absorbance peaks in UV spectra, which are very important parameters in determining the quality of vinegar, flow behaviour determining the viscosity and spectral fingerprint peaks were analysed at specific desired ranges.

It was observed that the absorbance values of the apple, red and yellow hawthorn vinegars have two peaks originated from organic acids and phenolic compounds. Absorbance peaks of vinegars between 190 nm and 240 nm wavelength are related to acetic acid concentration [41]-[43]. The main reason for the shifting of the peak values of the absorption coefficient resulting from the concentration of phenolic compound and acetic acid in the structure of vinegar to the higher energy region is related to the expansion of the energy gaps caused by the quantum confinement of organic acid molecules [50]. It was determined that the quantum confinement effect caused by the presence of organic acids causes a short release time [32], [51], [52]. From the lowest energy gap value calculated for yellow hawthorn vinegar, it was concluded that organic acids in its structure have a longer release time.

It was found that the flow behaviour of apple, red and yellow hawthorn homemade vinegars is compatible with the non-Newtonian behaviour defined as dilatant fluids (thickening liquids) flow. It was also determined that the flow curves of all vinegars behave in accordance with the Power law ($\eta = K\,\dot{\gamma}^{m-1}$) model [32]-[34], [53], [54]. The absorbance peaks around 3300 cm^{-1} and 1635 cm^{-1} wave numbers of the FTIR spectra for the samples were found to be related to the H-O-H asymmetric stretch vibration and H-O-H symmetric stretch vibration, respectively. Determining the mixture of acetic acid and water expected to be in organic vinegar from the spectral fingerprint peaks of vinegars was accepted as an important result [21], [57], [58]. From the spectroscopy and rheology analysis of apple red and yellow hawthorn vinegar, it was considered that these vinegars were good as commercial vinegars in the literature [23]. It was concluded that form UV spectra of yellow hawthorn vinegar has the highest acetic acid and phenolic compound concentrations. In this case, it can be said that yellow hawthorn vinegar has higher antioxidant and antibacterial effects compared to other apple and red hawthorn vinegars [8], [11], [12], [59].

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