

Optimal conditions for anthocyanins extracting from some food wastes

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ABSTRACT

The aim of this study is to determine the optimum conditions for anthocyanins extracting from the peels of eggplant, onion, red cabbage and pomegranate. The effects of solvent type, ratio of the raw material to solvent, temperature, pH, duration of extraction, and absorbance readings to the range of wavelengths 360-700 nm were examined. For the solvent types, the results showed that the best extraction solvent was ethyl alcohol acidified with citric acid at a concentration of 5% with a pH of 2, followed by ethyl alcohol acidified with hydrochloric acid, then the absolute ethyl alcohol in the third place, and the least was water and acidified water as an extraction solvent. The highest amount of pigment was found in pomegranate peels using ethyl alcohol acidified with citric acid, a value of 76.10 mg 100 g⁻¹ at a wavelength of 390 nm, while the water solvent exhibited the lowest amount extracted pigment of 5.44 mg 100 g⁻¹ at a wavelength of 540 nm in red cabbage peels. The optimum raw/solvent ratio for extraction was 40:1 weight (W)/ volume (V) in onion and eggplant peels with a value of 122.01 and 79.72 mg 100 g⁻¹, respectively, while the ratio of 1:20 (W/V) was the best for extracting both cabbage leaves and pomegranate peels. Temperature results indicated that the highest amount of anthocyanins is extracted at 60 °C for red cabbage, pomegranate and eggplant, with a value of 70.56, 96.21 and 118.43 mg 100 g⁻¹, respectively. On the other hand the temperature of 50°C was the best for extracting onion peels, with pigment amount of 122.01 mg 100 g⁻¹. Finally, the best time duration to extract the pigment is 60 minutes in all raw materials included in the study.

Article type: Anthocyanin, Extract, Food, Fruit, Wastes

Article type: Research Article.

INTRODUCTION

Colour is one of the characteristics of fruit and vegetable products. It provides information about the quality, safety, and freshness of food. Colour also gives an indication of the aesthetic and sensory values. Adding colours to food gives it an attractive and appetizing appearance (Shetty *et al.* 2017). Some companies used colours to attract a certain group of people, such as children. Colour is also used to improve the appearance of food products and their acceptance by the consumer. Sometimes colour is added to the manufactured products due to the loss of colour and flavour during manufacturing, especially when using high temperature or to hide the defects of low-quality products. Natural or industrial pigments and colours are also added to improve the original product colours in different production seasons, geographic region or for the purposes of ornamentation (Al-Hassan *et al.* 2006; Cortez *et al.* 2017; Alwan 2022; Shareif *et al.* 2022; Al-khaldy *et al.* 2022). Natural pigments are organic chemical compounds that absorb visible light in a range of wavelengths 350-750 nm. It contains natural compounds with healthy and safe benefits for human health. Its use dates back to 2600 BC, specifically in China. Natural pigments were extracted from plants such as fruits, seeds, roots, leaves, bark and trees, while the pigments were extracted from invertebrate animals, insects, algae, molluscs and microorganisms. It is a source of natural pigments, but due to its unavailability in large quantities, vegetable pigments have been relied upon to colour food products (Dyankova & Doneva 2016). Anthocyanins are plant pigments that are soluble in water and alcohol, and the most important group after chlorophyll that is visible to human eyes. It absorbs light

in a range of wavelengths and is characterized by a wide range of colours ranging from orange to pink and red to purple and blue depending on the molecular structure and pH (Al-Salihi *et al.* 2006; Ali *et al.* 2016). Anthocyanins consist of two six-carbon aromatic rings connected to a three-carbon heterocyclic carbon ring. The first ring A contains three double bonds and two hydroxyl groups, while the second C ring contains two double bonds and one oxygen atom, the third ring B contains three double bonds and one hydroxyl group followed by two side functional groups. R-groups determines the chemical compounds belonging to the anthocyanin pigments (Gerard *et al.* 2019). The most prevalent anthocyanins in nature are cyanidin, pelargonidin and delphinidin, which constitute 80% of leaf pigments, 69% of fruit pigments and 50% of flower pigments (Cortez *et al.* 2017). Therefore, the study is aimed to highlight plant residues which constitute a burden on the environment in order to produce high-value products in food industry, using natural colours to extract the anthocyanin pigments and also finding the optimal conditions for extraction to obtain the largest amount of anthocyanin pigments.

MATERIALS AND METHODS

Sample preparation

The raw materials were cleaned of dust and suspended matter. The red peels of the onions were removed and ground using an electric grinder (coffee grinder) to get a powder and placing in the polyethylene bags. The bags were tightly closed and kept frozen until further examinations. The eggplant and pomegranate fruits were washed with tap water, then dried. The eggplant fruits were peeled to obtain a thin layer of purple-coloured peels then left to dry overnight at the laboratory temperature, thereafter crushed and frozen in polyethylene bags until further use. In the case of the pomegranate only red peels were kept to be used and the yellow parts were excluded. The red peels were cut and ground in the mill to get a powder, froze in polyethylene bags until further use. The outer, inedible leaves of the red cabbage flower were removed, then it was cut into small pieces with a shredding machine, frozen in polyethylene bags until further examinations.

Anthocyanin pigment extraction

The method of Kuhestanya & Khodabandehlo (2014) was followed to extract anthocyanins with some modifications. Certain weight of the residues were placed separately with certain proportions of the solvent in Becker. The mixture was placed on a magnetic stirrer with a hot plate, while keeping the beaker nozzle closed. After stirring, the mixture was centrifuged for 20 min at a speed of $3835 \times g$. The process of re-extraction was repeated several times until the pigment was removed from the raw materials. The filtrate was taken and concentrated in a rotary evaporator at a temperature of 45°C . The absorbance was read in a range of positive lengths (360-700 nm) to determine the maximum wavelength. The pigment was calculated using the following equation, according to Ramadan & El-Hadidy (2015), on the basis of delphinidin 3- glucoside.

$$\text{Total anthocyanins (mg/100g)} = \frac{A \times DF}{W \times 55.9} \times 100$$

where W: weight of the sample; DF: inverted dilution; A: absorbance

Factors affecting the percentage of anthocyanin pigment quotient

Type of solvent

Five types of solvents were used to investigate their effects on the percentage of the pigment yield. The first solvent was water. The second included water acidified with hydrochloric acid at a concentration of 1% in a ratio of 40:60 (V:V). The third was absolute ethyl alcohol, the fourth, acidified ethyl alcohol with a concentration of 1% hydrochloric acid in a ratio of 40:60 (V:V) and the last, ethyl alcohol acidified with citric acid at a concentration of 5% at a ratio of 40:60 (V:W). The ratio of the starting material included solvent: 30:1 (W:V), temperature 50°C , pH 2 and a period of one hour for extraction. The same extraction method was followed in the previous paragraph, however, the amount of pigment was calculated from the above equation.

Raw material / Solvent ratio

The effect of the ratios of the starting material to the solvent that can give the highest possible pigment is studied using the following ratios: 1:10, 1:20, 1:30, 1:40 and 1:50 (W:V). Using the same method in extracting the pigment. The following factors, starting material, the temperature, the pH and the time period, were changed for each type of solvent and the resulting amount of pigmentation was calculated as before.

Temperatures

Different extraction temperatures were tested to obtain the highest yield of 30, 40, 50, 60, 70 and 90 °C.

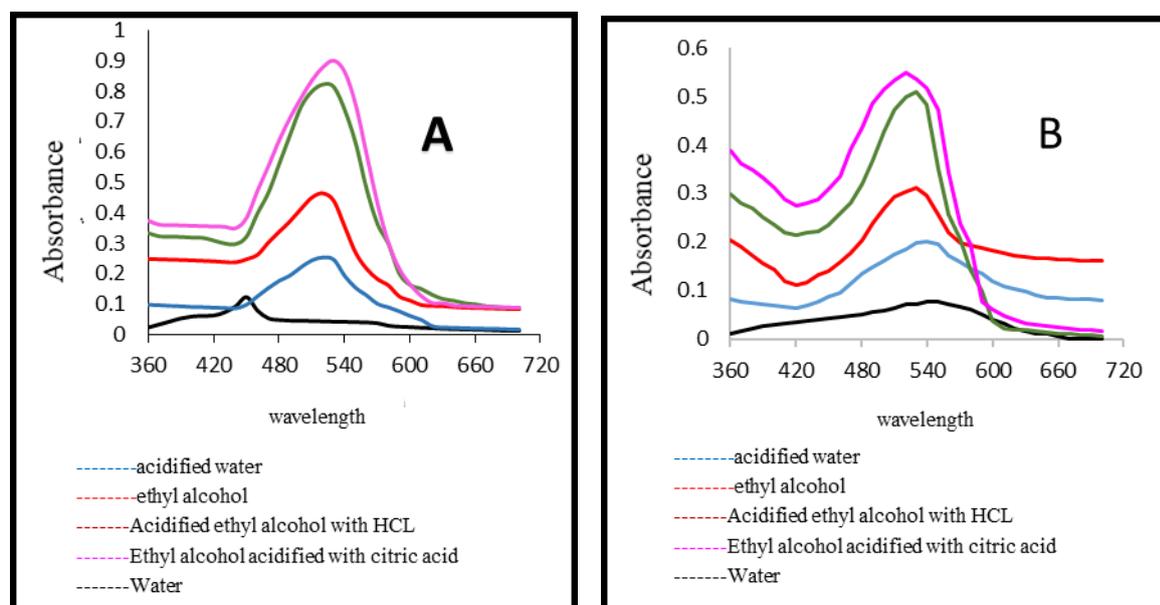
Duration of extraction

A series of preliminary experiments was conducted to select the best extraction time that gives the highest pigment yield 30, 60, 90 and 120 min, by keeping the above factors unchanged. The pigment was extracted and kept in opaque, freeze-tight containers for subsequent experiments.

RESULTS AND DISCUSSION

Fig. 1 shows the maximum wavelengths when extracting anthocyanins from purple eggplant peels, red cabbage leaves, red onion peels and pomegranate peels using different types of solvents. The acidified ethyl alcohol with citric acid gave the highest absorbance in all the raw materials. The maximum wavelength of the red onion peels extract was 530 nm with a value of 0.898, exhibiting the highest absorbance, while the maximum wavelength in the eggplant peels was 540 nm with a value of 0.65. The greatest wavelength in cabbage leaves, displaying the highest absorbance of 520 nm, was 0.55. In the case of pomegranate peels, the maximum wavelength for extracting anthocyanins was 390 nm with an absorbance of 0/0635.

The lowest absorbance for all raw materials resulted by using water as a solvent. The maximum wavelength of onion was 450 nm, revealing the highest absorbance, while those of eggplant and cabbage were 530 and 540 nm with absorbance of 0.125, 0.125 and 0.152 respectively. The pomegranate exhibited an absorbance of 0/711 at a wavelength of 410 nm. Depending on the type of solvent and the type of starting material, the maximum wavelength ranges from 500-540 nm for pigments extracted from cabbage leaves and eggplant peels, 390-410 nm for pomegranates, and 450-530 nm for onion. The anthocyanin pigment is extracted from plants with ethyl alcohol to obtain a stable pigment-positive flavylium ion in an acidic media. Because ethyl alcohol is the best acidifying agent, it works with the pigment as an ionic coupling agent and also due to low polarity, it functions by exchanging charges to remove pigment from the plant with a value of 4.3, while the water's polarity is estimated at 10 (Perez *et al.* 2021). Citric acid is considered to be a weak organic acid that is nutritionally safe. Organic acids work to prevent the pigment from decomposing during extraction by not removing the acyl group linked to sugar (Shetty 2017).



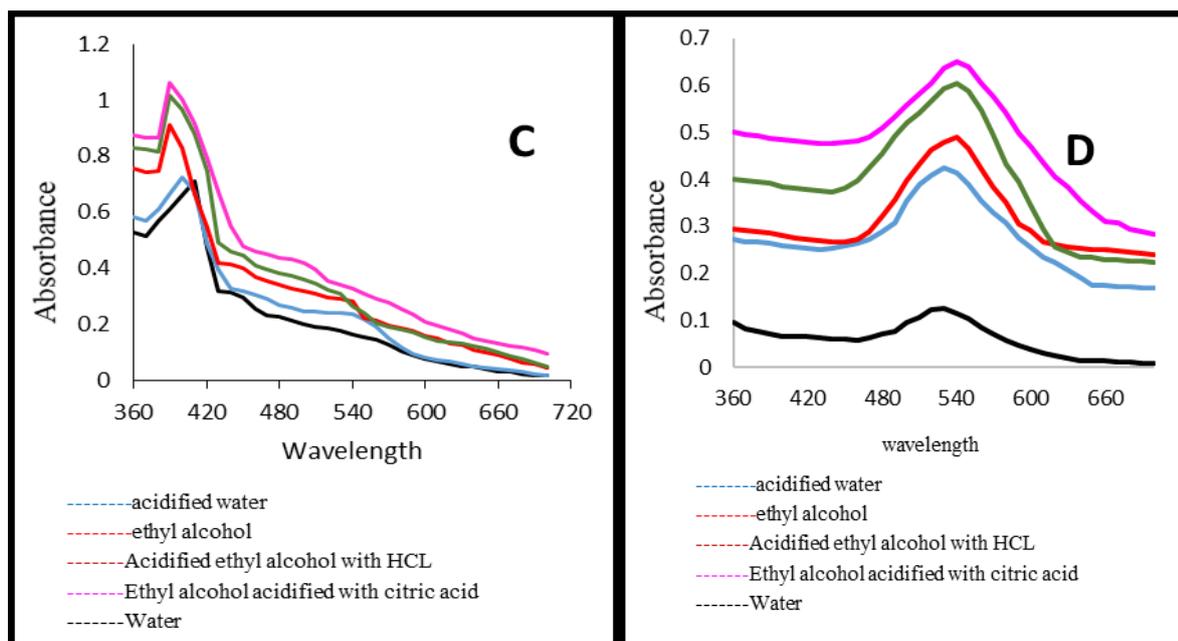


Fig. 1. The effect of the type of solvent in determining the maximum wavelength of anthocyanin pigment extraction. **A:** Onion peels, **B:** Red cabbage leaves, **C:** Pomegranate peels, **D:** Eggplant peels.

These results supported what was reported by Filip *et al.* (2012) who studied the anthocyanins pigment extracted from 8 types of strawberries, they showed that there is a difference in the maximum wavelengths according to the plant variety, which ranged between 520-546 nanometers. The results also supported what was mentioned by Gerard *et al.* (2019) who extracted anthocyanins from three different types of raw materials: sweet tomato, black carrot and grape juice, and the wavelengths were 527, 522 and 529 nm. The total amount of anthocyanins at the maximum wavelength was shown in the statistical analysis in Table 1, and there were significant differences when employing the solvents examined. The acidified ethyl alcohol solvent at a concentration of 5% citric acid exhibited the highest amount of pigment in pomegranate peel extract amounting to 76.10 mg/100 g with a significant difference from the rest of the other solvents in all the samples examined. This is followed by the extract of onion peels of 64.30 mg/100 g, while the lowest result obtained in the extract of red cabbage leaves with the amount of 39.21 mg /100 g of the same solvent. The lowest amount of pigment was observed when using water solvent in red cabbage leaf extract, with a significant difference of 5.44 mg/100 g from the rest of the examined samples. The total amount of anthocyanins, using acidified ethyl alcohol at a concentration of 1% hydrochloric acid, ranged from 36.42-72.81 mg/100 g in all the examined raw materials.

Table 1. Anthocyanin pigment concentrations (mg 100 g⁻¹) using different solvents of plant materials.

| Raw materials | Water | Acidified water with HCl (1%) | Ethyl alcohol | Acidified ethyl alcohol with HCl (1%) | Acidified ethyl alcohol citric acid (5%) | L.S.D _{0.05} |
|---------------|-------|-------------------------------|---------------|---------------------------------------|--|-----------------------|
| Onion | 8/95 | 18/14 | 33/35 | 58/86 | 64/3 | 0.2641 |
| Red cabbage | 5/44 | 14/38 | 22/26 | 36/42 | 39/21 | 0.3690 |
| Pomegranate | 50/88 | 51/70 | 65/23 | 72/81 | 76/10 | 0.1795 |
| Eggplant | 8/95 | 30/41 | 35/06 | 43/22 | 46/44 | 0.1866 |

The difference in the amount of pigment resulted between the raw materials is due to the nature of these pigments that contains hydrophilic part represented by hydroxyl groups and the main positive charge, and another non-hydrophilic part, which contains multiple phenols that dissolve in organic solvents. Accordingly, it is preferred to use mixtures of solvents in the extraction. Anthocyanins are found in plant tissue cells under the epidermis dissolved in the cell sap within vacuoles associated with cellulose, hemicellulose, lectin and pectic substances. The purpose of organic acid is to break the hydrogen bonds between these compounds, thus, the selective permeability is destroyed and the intracellular osmotic pressure disturbance occurs, which leads to the soluble substances leaving the cell easily (Perez *et al.* 2021).

These results supported what was stated by Lozovskaya *et al.* (2012) that estimated the amount of anthocyanins from red onion peels using water and acidified methanol solvents. The water exhibited the lowest amount of pigment of 8 mg/100 g at a temperature of 50 °C. The results were also consistent with the findings of Selim *et al.* (2008) who studied the effect of four solvents on the concentration of anthocyanin pigments extracted from Roselle. Their results showed that water displayed the lowest amount of pigment of 576 mg 100 g⁻¹, while ethyl alcohol acidified with hydrochloric acid gave 1457 mg 100 g⁻¹. According to the results depicted in Fig. 2, shows that the best ratio of raw material to solvent in determining the maximum wavelength extracting anthocyanins is 40:1 (W:V) in extracts both onion peels and eggplant which exhibited the highest absorbance of 1.705 and 1.114 at wavelength 530 and 540 nm with significant differences in the total amount of extracted anthocyanins. The highest amount of extracted anthocyanins was found in onion peel extracts with a significant difference from the rest of the other extracts of 122.01 mg 100 g⁻¹ (Table 2). In the case of pomegranate and cabbage leaves, the ratio of 20:1 (W:V) raw material to solvent exhibited the highest absorbance and the highest amount of total anthocyanins, i.e., 2.519 and 0.773, 90.13 and 55.32 mg 100 g⁻¹. The least amount of pigment was found in the extract of eggplant peels at a ratio of raw material: solvent 10:1 of 18.605 mg 100 g⁻¹ with a significant difference from the rest of the other extracts.

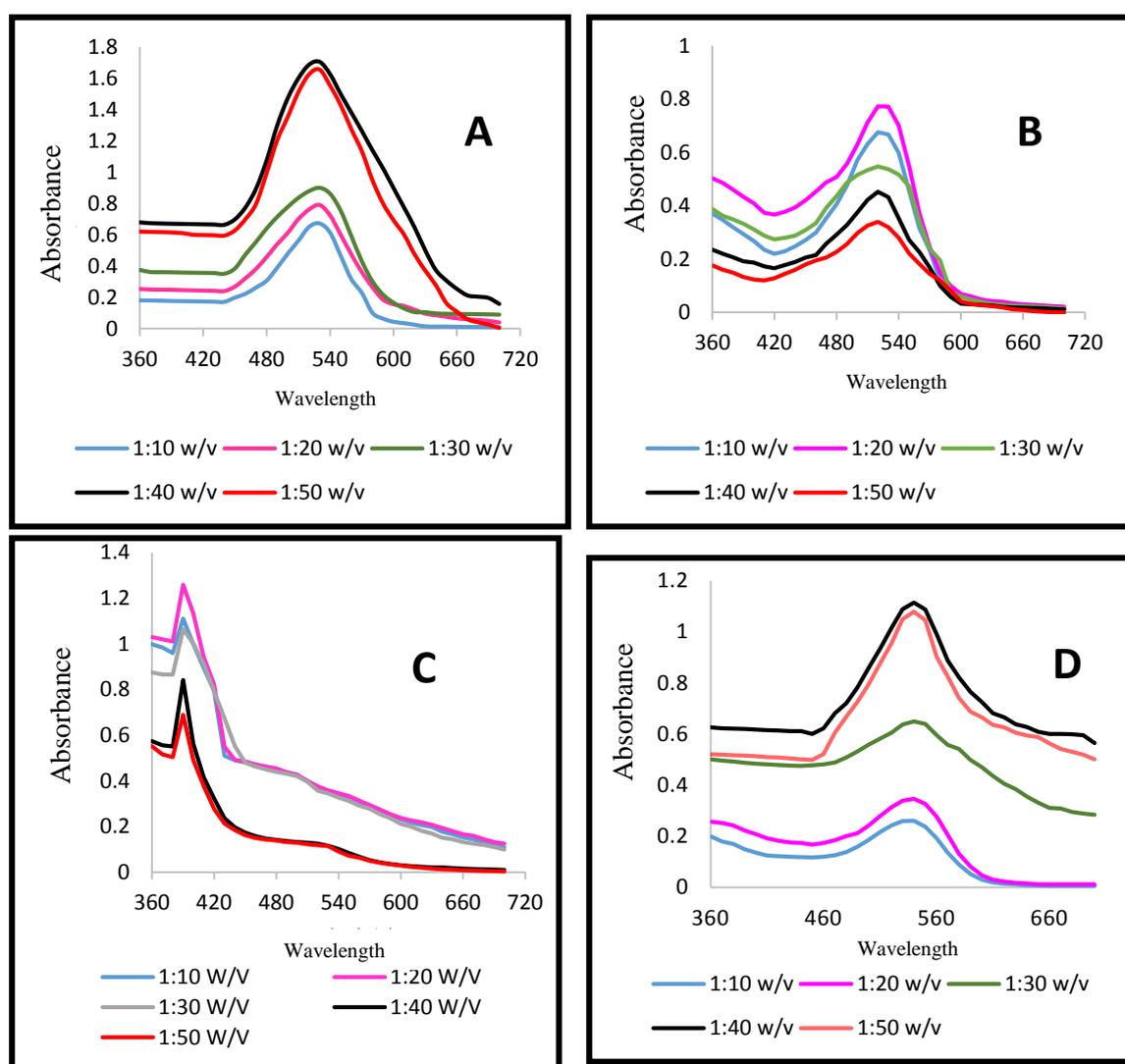
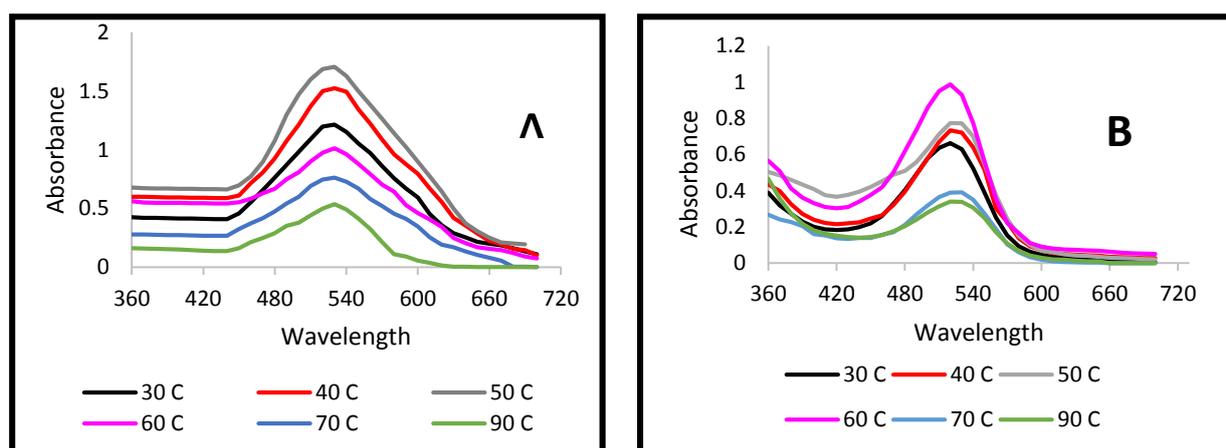


Fig. 2: Effect of a raw material: solvent ratio in determining the maximum wavelength of anthocyanin pigment extraction. **A:** Onion peels, **B:** Red cabbage leaves, **C:** Pomegranate peels, **D:** Eggplant peels.

Table 2. Anthocyanin pigment concentrations ($\text{mg } 100 \text{ g}^{-1}$) using different proportions of the raw material:solvent.

| Raw materials | 10: 1 | 20: 1 | 30: 1 | 40: 1 | 50: 1 | L.S.D _{0.05} |
|---------------|--------|-------|--------|--------|--------|-----------------------|
| Onion | 48/09 | 56/50 | 64/29 | 122/01 | 118/43 | 0/3730 |
| Red cabbage | 48/37 | 55/32 | 39/213 | 32/35 | 24/26 | 0/2980 |
| Pomegranate | 79/5 | 90/13 | 76/10 | 60/25 | 49/30 | 0/4633 |
| Eggplant | 18/605 | 56/50 | 46/422 | 79/72 | 77/21 | 0/3872 |

The reason was that the grinding process caused damage to the plant material cell walls by reducing the size of the particles and thus increasing the surface area for the solvent contact with the pigment and allowing the solvent to penetrate into the cell, which leading to improve the extraction process (Silva *et al.* 2017). It is obvious from Fig. 2 and Table 2 that by elevating the ratio of the raw material: the solvent in all the extracted plant materials led to a drop in the amount of extracted anthocyanins, which was attributed to the fact that a large amount of solvent needs a longer time for extraction (Le *et al.* 2019). The above results supports the information found by Le *et al.* (2019) who used different ratios of the raw material: solvent 2:1 - 5:1 (W/V) when extracting anthocyanin pigments from a kind of fruit, *Carissa carandas* L. The ratio of 3:1 exhibited the highest amount of pigment (7.28 mg g^{-1}), while 5:1 showed the lowest amount (6.84 mg g^{-1}). The results did not support the results found by Todaro *et al.* (2009) once estimating anthocyanins from eggplant peels using different ratios of the raw material: solvent 10:1 - 80:1 (W/V). Their results showed a direct relationship between the ratio of raw material: solvent and the total amount of pigment using ethyl alcohol solvent, while the stability of the total amount of pigment was obtained when using tartaric acid 20:1 -80:1. Fig. 3 shows the effect of extraction temperatures of 30-90 °C in determining the maximum wavelength for extracting anthocyanins from the plant materials examined. The temperature of 60 °C resulted in the best extraction from each of the eggplant peels, red cabbage leaves and pomegranate peels at the wavelengths of 540, 520 and 390 nm, with a total amount of anthocyanins of 118.43, 70.56 and 96.212 $\text{mg } 100 \text{ g}^{-1}$, with significant differences between the three extracts. The temperature of 50 °C led to the highest amount of pigment ($122.01 \text{ mg } 100 \text{ g}^{-1}$) from the red onion peels extract at the wavelength of 530 nm with a significant difference from other extracts (Table 3). This elevation in the amount of pigment can be attributed to its high solubility and the speed of transformation from inside the cell to the outside by breaking down the cell walls, and this depends on the type of solvent and raw materials used (Silva *et al.* 2017; Perez *et al.* 2021).



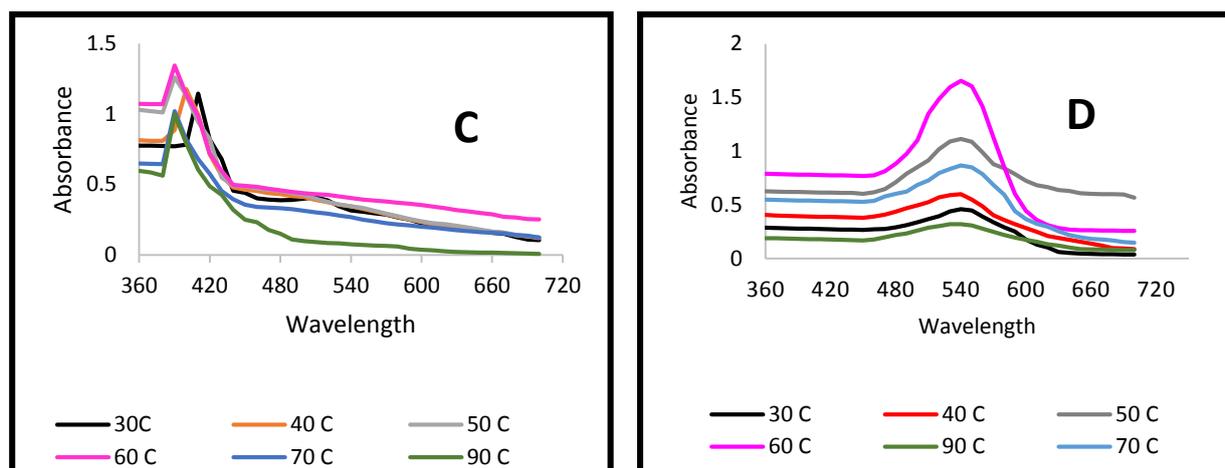


Fig. 3. Effects of temperature on the anthocyanin pigment extraction to determine the maximum wavelength. **A:** onion peels, **B:** red cabbage leaves, **C:** pomegranate peels., **D:** Eggplant peels.

Table 3. Anthocyanin pigment concentrations (mg 100 g⁻¹) using different temperatures of the studied plant materials.

| Raw materials | 30 °C | 40 °C | 50 °C | 60 °C | 70 °C | 90 °C | L.S.D _{0.05} |
|---------------|-------|--------|--------|--------|-------|-------|-----------------------|
| Onion | 86/77 | 108/95 | 122/01 | 72/74 | 54/46 | 38/32 | 0.0848 |
| Red cabbage | 47/44 | 52/45 | 55/32 | 70/56 | 27/84 | 24/33 | 0.4583 |
| Pomegranate | 81/86 | 84/26 | 90/13 | 96/212 | 72/99 | 71/38 | 0.3658 |
| Eggplant | 32/77 | 42/93 | 79/72 | 118/43 | 62/04 | 22/93 | 0.0544 |

At extraction temperature of 70 °C the amount of pigment ranged from 27.84-72.99 mg/100 g, while lowest amount of pigment is found at 90 °C which exhibited the lowest amount of pigment from the eggplant peel extract (22.93 mg/100 g). This resulted in significant difference than the all extracts, i.e., onion peels, cabbage leaves and pomegranate peels (38.32, 24.33 and 71.38 mg/100 g respectively). High temperatures cause the pigment to quickly break down in the crusts, thus, a decrease in the amount of pigment extracted by two mechanisms, first is the hydrolysis of the 3-site glycoside in anthocyanins. The formation of anthocyanidin, which has an unstable effect, and then the hydrolysis of the heterogeneous beryllium ring of anthocyanidins, producing the form of chalcone responsible for the brown colour, and then decomposing it to the benzyl compound (Sharif *et al.* 2010; Askar *et al.* 2015). These results support the findings of Perez *et al.* (2021) who studied the effect of temperature on the total pigment quantity of a grape variety, *Vaccinium floribundum* using temperatures of 30 and 60 °C. They reported that the highest amount of pigment extracted using a temperature of 60 °C was 335.49 mg/100 g, while 30 °C exhibited the least amount (187.94 mg/100 g). The results were also in line with those of Liu *et al.* (2012) who used three temperatures of 55, 65 and 75 °C, to extract anthocyanin pigments from one of the rose varieties, *Rhodomyrtus tomentos* for 120 min. Their results showed that 65 °C is the best temperature in extracting the highest amount of the pigment (4.313 mg g⁻¹), while the least amount (3.730 mg g⁻¹) resulted using 75 °C. The results were also in accordance with those of Le *et al.* (2019) who studied the effect of temperatures from 30 to 80° C on the extraction of total anthocyanin pigments from a type of fruit *Carissa carandas* L. They were found that the best extraction temperature was 50°C with the amount of 6.67 mg g⁻¹, while 80°C resulted in the lowest amount. Fig. 4 shows the time required to extract the anthocyanin pigments from the plant materials examined and determining the wavelength that exhibits the highest concentration of the pigment. It was observed that there was an increase in the pigment concentration after 60 min compared to 30 min: 112.01, 70.56, 96.21 and 118.43 mg 100 g⁻¹ for each extract from red onion peels, red cabbage leaves, pomegranate peels and eggplant peels.

Table 4. Anthocyanin pigment concentrations ($\text{mg } 100 \text{ g}^{-1}$) from plant materials using different time periods.

| Raw materials | 30 min | 60 min | 90 min | 120 min | L.S.D _{0.05} |
|---------------|--------|--------|--------|---------|-----------------------|
| Onion | 96/96 | 122/01 | 82/86 | 57/25 | 0/2564 |
| Red cabbage | 59/9 | 70/56 | 55/24 | 32/42 | 0/2314 |
| Pomegranate | 84/54 | 96/21 | 90/27 | 78/68 | 0/2865 |
| Eggplant | 107/27 | 118/43 | 85/87 | 60/29 | 0/0365 |

The alterations in the anthocyanin pigment concentrations ($\text{mg } 100 \text{ g}^{-1}$) according to the different times of extraction at the maximum wavelengths of 530, 520, 390 and 540 nm are presented in Table 4. The concentration of the extracted pigments was found to be dropped over time. The lowest value was found after 120 min of extraction which was due to the breakdown of the pigments by the upraised extraction time at high temperatures. There was a relationship between the time used for extraction and the temperature and also the solvent used (Nabli *et al.* 2013; Le *et al.* 2019).

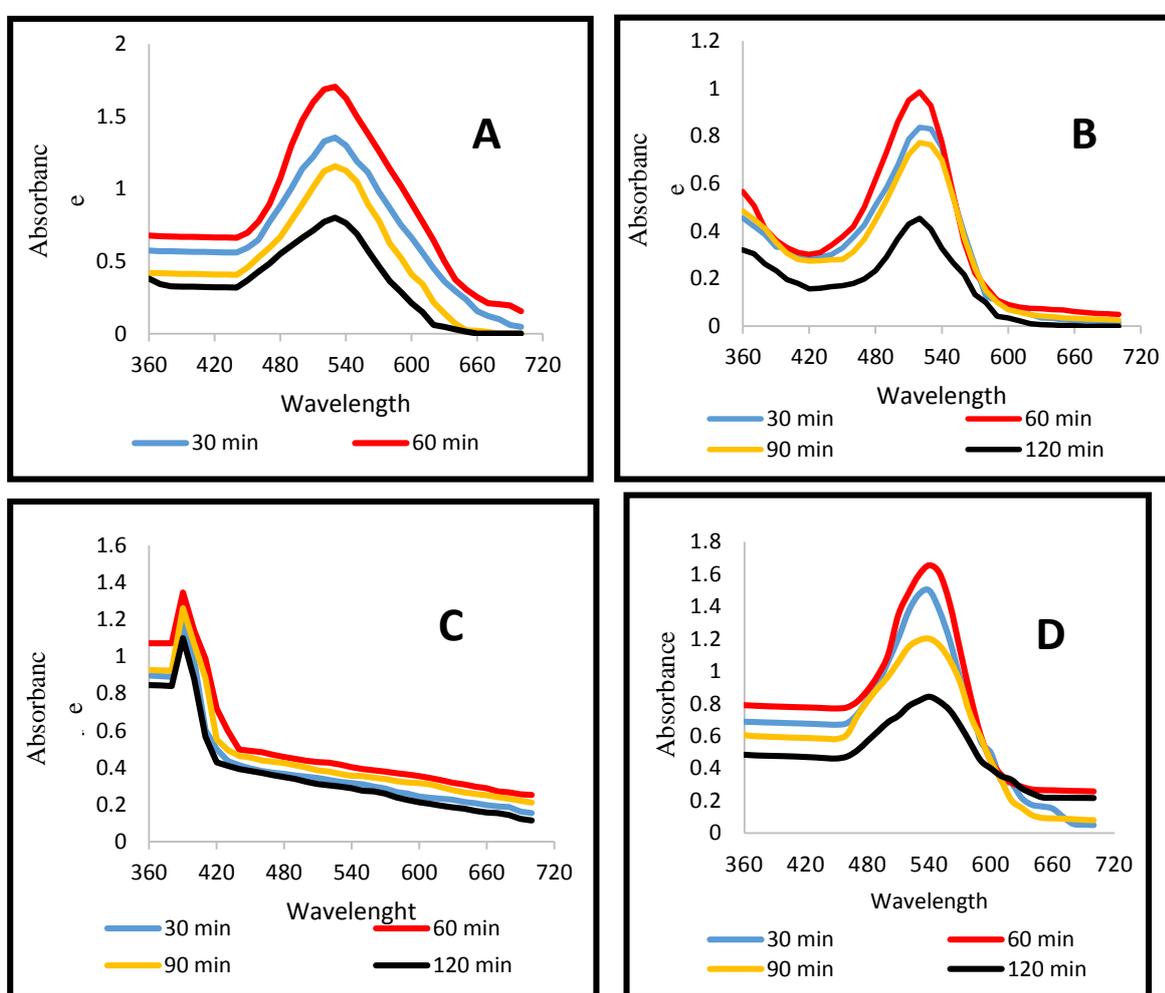


Fig. 4. The effect of the time period for determining the maximum wavelength of anthocyanin pigment extraction. **A:** Onion peels, **B:** Red cabbage leaves, **C:** Pomegranate peels, **D:** Eggplant peels.

The results were in agreement with what was found by Le *et al.* (2019) when examining the effect of the extraction times of 15-90 min. Their results exhibited that 45 min resulted in the highest amount of pigment ($6.67 \text{ mg } \text{g}^{-1}$), while decreased to $5.65 \text{ mg } 100 \text{ g}^{-1}$ after 90 min. The results were also in line with the findings of Nabil *et al.*

(2013) who estimated the amount of pigment using two periods of 3 and 6 hours for extraction from a grape variety. The best duration of extraction was 3 h compared to 6 h (6.77 and 6.53 mg 100 g⁻¹, respectively).

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