Research Article

Investigation of removal of anthocyanin in turnip juice wastewater by using different adsorbents

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Abstract
The aim of this study is to eliminate the colour pigment of anthocyanin in the composition of turnip juice under different experimental conditions using low cost adsorbents (eggshells, pumpkin seed hulls, almond shells, black–green–red tea wastes, natural clay material). For this purpose, a series of discrete adsorption experiments were performed. Contact time for each set of experiments, adsorbent amount, mixing speed and temperature were fixed at 30 min, 2 g, 150 rpm and 20 °C. The effects of pH change of wastewater (acidic-neutral-basic) on colour removal and adsorption process were examined. The most important advantage of using such adsorbents is their ability to solve colour pollution problem as effective and economical adsorption. As a result of the studies, the colour parameter of the anthocyanins was removed from the wastewater at a low rate (14.08%).

Keywords Adsorption · Adsorbent · Anthocyanin · Colour · Turnip juice · Wastewater

1 Introduction

Since the fermentation increases the taste, aroma, composition, nutritive value and shelf life of nutrients, it is possible to find fermented products (cheese, yoghurt, pickles, kefir, boza, tarhana and turnip juice) in every society [1–3]. Turnip juice is one of the fermented products to be produced locally and is defined as an extract, purple carrot (Daucus carota), turnip (Brassica rapa) and if desired, a product that is obtained by mixing turnip juice, bulgur flour, sour paste [4, 5]. Turnip juice produced by lactic acid fermentation is a red-coloured, sour-tasting and fuzzy beverage and the basic raw material used in the production is stated as purple carrots. The turnip juice is produced in Adana, Mersin, Gaziantep, Hatay, Kahramanmaraş and Osmaniye provinces in Turkey [6]. The unique colour of the turnip juice comes from the anthocyanins in the composition of purple carrot, which is the main material. It is stated that the anthocyanin content of turnips juice is between 94 and 238 mg/L [7]. Turnip juice is of great importance for health. Therefore, there appears to be an emerging industry in Turkey [6, 8].

There is no standard flow chart in the production of turnip juice, mainly due to its traditional production. Two different methods are applied to the production of turnip juice. Traditional turnip juice production takes place in two stages (Fig. 1). The first phase is the phase where the dough fermentation takes place, and lactic acid bacteria are enriched at this stage. In the second phase, carrot fermentation takes place and during fermentation, anthocyanins pass into the fluid. 15–20% solids content of turnip juice consists of approximate size 2 cm turnip pieces [9, 10]. With this method, the fermentation is carried out at single stage and at room temperature by adding 20% purple carrot, 3% bulgur flour, 1% salt, 0.2% bread yeast and water to the fermentation tank in the production of turnip juice (Fig. 2) [11–13]. Purple carrot is a rich source of anthocyanin, the main ingredient used in the production of turnip juice. Purple carrots are mostly grown in Turkey, Afghanistan, Egypt, Pakistan and India. Ereğli district in Konya is the main production region of purple carrot in

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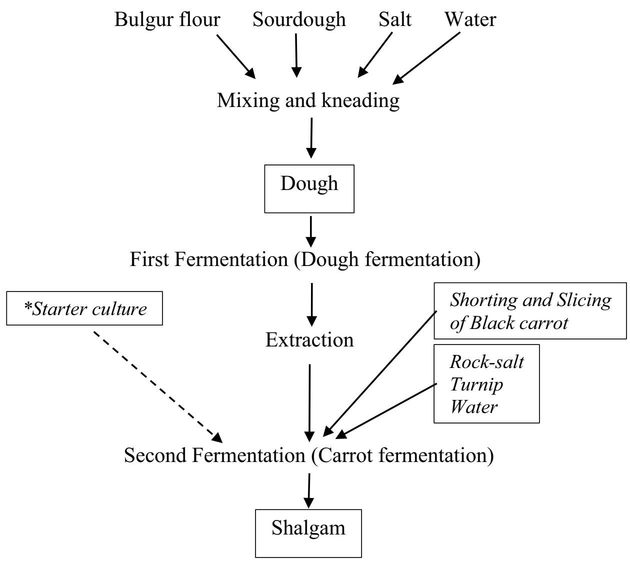

Fig. 1 Turnip juice production by traditional method [11]

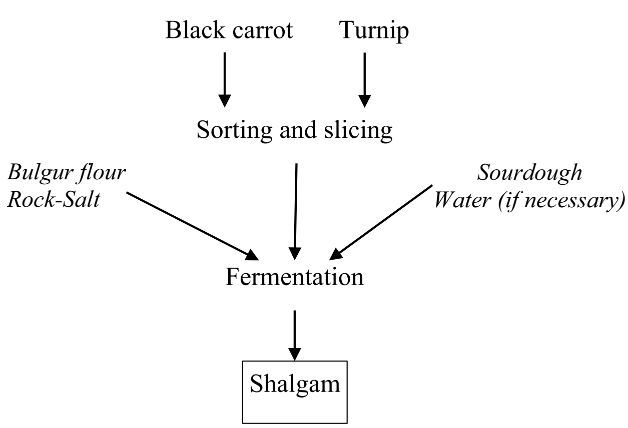

Fig. 2 Turnip juice production by direct method [11]

Turkey [10, 14, 15]. Purple carrots contain a high amount of anthocyanin that is found to be fresh in 1750 mg/kg [16, 17].

The main anthocyanins to be found in purple carrots are cyanide-based, cyanide-3-xylosyl-lukoil-galactoside, cyanide-3-xylosyl-galactose, cyanide 3-xylosyl-glycosinolate derivatives as synoptic ferulic and coumaric acid derivatives of the form (Fig. 3) [18–21]. Anthocyanins have become increasingly important today because of their natural colouring material for the food industry [22, 23]. According to the numbering system used by the Codex Alimentarius Commission, anthocyanins are listed as natural colouring by European Union legislation and are encoded as E163 [18, 24, 25].

Industrial wastewater differs greatly from domestic wastewater in character, source and quantity. Industrial waste must be controlled by factors that cause pollution of the environment and treated to prevent the use of used water of industrial facilities from contaminating the natural water environment. In particular, colour waste is usually caused by industrial activities, and the textile, leather, food, paper industries are the most common industries to be found. These coloured wastes can cause problems both in the provision of discharge parameters and in the environmental absorption. In addition, it is a known fact that it will cause physical view and odour pollution. In the production of turnip water, coloured wastewater is formed for the washing of carrots and tanks. A series of processes have been developed and studied for the treatment of such wastes that give colour to the receptor environment. Some of them are coagulation and aggregation, ozone, membrane-type filtration, photocatalytic oxidation, electrochemical and biological treatment [26]. However, many of these methods limit the high operating costs and excessive use of sludge production. Adsorption is a method applied successfully and effectively to remove the dyes from the waste [27, 28]. Low cost, high efficiency, easy applicability, the ability to minimize biological or chemical wastes, lack of extra substances, adsorb and the possibility of adsorbing recovery are some of the key advantages of adsorption [29, 30]. There are many agricultural and forest-based products that can be used as adsorbent [31, 32]. In this study, the chemical profile of the turnip water produced by fermentation was evaluated in accordance with the literature and the removal of the anthocyanin colour pigment in the structure of turnip water and in the wastewater content after production was investigated using different adsorbents. This research was carried out in wastewater laboratories of Aksaray University Environmental Engineering Department between January and February of 2019 for 2 months. The most important difference of this study compared with others is that when examined in previous years, no studies have been found using the adsorption process of the colour parameter of turnip juice production waste. Moreover, compared with others, the most important difference of this study is to use the batch experimental operation without changing the adsorbents. There are some studies in the literature to remove different synthetic dyes with modified adsorbents. However, there are not many studies on natural dyes. In this study, experiments were performed with natural adsorbents instead of modified adsorbents. In order to extract anthocyanin from wastewater, the study used evaluable waste eggshells, pumpkin seed shells, almond shells, black–green–red tea wastes, natural clay material as potential adsorbents. These adsorbents have been selected as waste and garbage due to their high amount of forms coming from homes, workplaces and

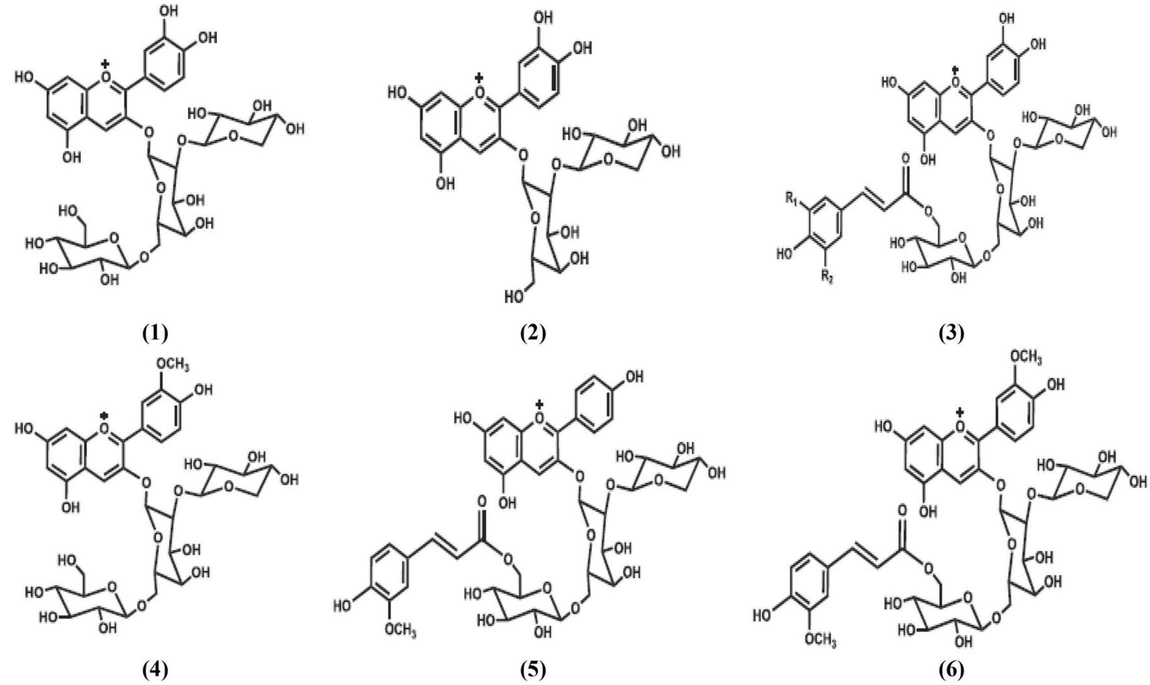

Fig. 3 Anthocyanin groups in the purple carrots [18, 21]: (1) cyanidin 3-xylosyl-glucosyl-galactoside, (2) cyanidin 3-xylosyl-galactoside, (3) sinapic acid derivative of cyanidin 3-xylosylglucosylgalactoside, (4) peonidin 3-xylosyl-glucosyl-galactoside, (5) ferulic acid derivative of pelargonidin 3-xylosyl-glucosyl-galactoside, (6) ferulic acid derivative of peonidin 3-xylosyl-glucosyl-galactoside

industries. The application of these selected materials has two advantages: First, they can perform as an effective and economical adsorbent to dissolve colour contamination. Another advantage is that these wastes, which are accepted as garbage all over the world, can be used in a powerful and beneficial way. Thus, sustainability in terms of both environmental and wastewater treatment is handled in an adequate and cost-effective manner.

Table 1 Some features of turnip juice wastewater

Parametre	Unit	Concentration
pH	–	5.72
Conductivity	µS/cm	1523.00
Turbidity	NTU	7.00
Suspended solids	mg/L	80.00
Biological oxygen demand (BOD_5)	mg/L	535.00
Chemical oxygen demand (COD)	mg/L	764.70

2 Methodology

2.1 Wastewater characterisation

The wastewater sample used in the study was taken from the washing water used for tank cleaning of a plant producing turnip water in Ereğli district of Konya. Characterization analysis of wastewater was carried out in the Environmental Engineering Department of Aksaray University. For the experiments, the samples were brought to the laboratory within 24 h by cold chain and analysed at room temperature. In the study of wastewater samples, the methods accepted and proposed in the whole world and applied frequently in Turkey were used [33]. As a result of the experiments carried out, the results of the analysis of raw wastewater samples are given in the Table 1.

2.2 Chemical compounds and microbiological properties of the turnip juice

The chemical and physical properties of turnip water should be as in Table 2 in accordance with TS 11149 turnip water standard [34]. The pH value of turnip water in accordance with the standard of turnip water 3.3–3.8, titration acidity in lactic acid 4.5–5.5 g/L, soluble dry matter at least 2.5% and salt ratio 2% at most. The amount of water-soluble in water consists of carbohydrates, salt, organic acids and minerals. In turn, there are trace amounts of saccharose, glucose and fructose sugars.

Table 2 The values of turnip juice specified in TS 11149 standard [34]

Parameter	Unit	Concentration
Total acidity (in terms of lactic acid)	g/L	≥ 6.0
pH	–	3.3–3.8
Lactic acid	g/L	4.5–5.5
Volatile acid (as acetic acid)	g/L	0.7–1.2
Dry substance	%	2.5
Ash (w/v)	%	2.0
Salt (NaCl, w/v)	%	2.0
Colour	pH = 1.0	Red–purple
	pH = 7.0	Gray–green

The microbiology of the turnip water is complicated and it is not known in details. The presence of the microorganisms having probiotic proerties such as *L. plantarum, L. helvaticus, L. reuteri, L. fermentum, L. buchneri, L. helvaticus, L. reuteri, Saccharomyces cerevisiae, Lactococcus, Pediococcus, Leuconostoc* and *L. delbrueckii* were found in the studies to be conducted [35–38].

2.3 Adsorbent preparations

Almond shell, egg shell, banana shell, pumpkin seed shell, Rooibos (red)–green–black tea waste, clay were used as the absorbents. The wastes used in the research were collected from local markets and houses. Prior to using these materials as adsorbs, the waste washing process was performed several times with pure water for 24 h at room temperature to remove any dirt particles that might be present on the surface. After washing, all adsorbents have dried in the air oven at 100 °C for 24 h (Memmert GmbH Co. KG, Germany). Later, washed and dried adsorbents were crushed using the Retsch GM 200 brand laboratory mill. Prior to adsorption experiments, no chemical or physical process was applied. In this study, almond shell, eggshell, banana shell, pumpkin seed shell, Rooibos (red)-green-black tea waste, clay minerals used were milled and used in powder form.

2.4 Devices and chemicals

All chemicals are analytical (more than 97% purity) and have been purchased from Merck GmbH (Darmstadt, Germany). The pH of each solution is set to the desired value using a pH meter with the addition of 0.01 M NaOH or 0.01 M HC1 drops if necessary. For batch adsorption experiments, ZHWY-200B, ZHICHENG analytical model thermal agitator (150 rpm shake rate) was used. Turnip juice wastewater, after sedimentation 0.45 µm pore-sized membrane filters is filtered. Colour analysis was performed using the Perkin Elmer Optima 2100DV model UV/Vis spectrometer. The pH and conductivity measurements were made with a combination electrode with LABQUEST2 digital ion analyser. The COD, BOD_5, TDS analyses were performed as indicated in the standard methods [33].

2.5 Characterization of adsorbents

The detailed composition of almond shell, eggshell, banana peel, pumpkin seed hull, Rooibos (red)–green–black tea waste, clay minerals are shown in Table 3 [39–51]. The surface physical morphology of the adsorbents identified by scanning electron microscope (SEM) was evaluated according to previous studies due to lack of laboratory facilities. Scanning electron microscope observations of adsorbents can be seen in Fig. 4 [39–51]. It can be seen that all adsorbents were agglomerated and have an irregular round shape. Also, all adsorbents have many large and medium pores. Through these pores, it is easy to catch and adsorb the dye.

2.6 Test of adsorbents

Adsorption studies were carried out at 20 °C temperature with the help of mechanical agitation in 100 ml conical flasks by adjusting the contact time for each set, adsorb amount, mixing speed and temperature to remain constant. In order to examine the effect of pH on anthocyanin, the pH tests were carried out using data in optimum conditions by taking 50 ml of all adsorbents and solutions set in specific pH values. Due to the negative effect of the temperature on the anthocyanin, all the experiments were conducted at room temperature. When determining the colour parameter, the European norm is based on the standards set according to DIN ISO 7887, the colour measurements were measured at 436–620 nm with visible light Novespec II Spectrophotometer measurements. The aim of using 3 different wavelengths in colour measurement with visual colour number (RES) method is to produce yellow and shades in 400–500 nm band, red and shades in 500–600 Nm band, Blue and shades in 600–700 nm band. These measured values are calculated by replacing them in an Eq. 1 below. A, the absorbance value of the sample in λ wavelength; d, bathtub thickness (mm); f, spectral absorption value (f = 1000); res (λ), λ wavelength number of colour (res) value (1/m)

$$\mathrm{RES}\,(\lambda) = \frac{\mathrm{A}}{\mathrm{d}} \times \mathrm{f} \tag{1}$$

2.7 Anthocyanins

The chemical structure of the anthocyanins investigated in the study and the other groups found in the common form in nature is shown in Fig. 5 [52–57]. Anthocyanins

Table 3 Some properties of adsorbents used in experimental studies

Properties	Moisture (wt%)	Volatile matter (wt%)	Ash content (wt%)	Carbon (wt%)	Hydrogen (wt%)	Nitrogen (wt%)	Oxygen (wt%)	Sulfur (wt%)	Surface area (m^2/g)	Porosity (cm^3/g)	References
Almond shell	13.70	81.10	0.94	49.70	6.30	0.26	42.79	0.01	967	0.49	[41, 45, 47]
Pumpkin seed hull	7.60	70.97	3.90	48.79	7.52	3.97	39.72	< 1	645	0.96	[40, 43, 49]
Banana peel	9.80	85.26	5.01	40.24	6.14	1.30	52.22	0.098	220	1.23	[43, 47, 50]
Clay Minerals	3.3	21.7	24.5	79.7	4.36	2.12	12.1	1.55	20.7	0.27	[48, 50]
Egg shell	0.5	–	43.5	13.09	0.35	0.54	29.46	0.03	1.023	0.0065	[39, 46]
Black-Green-Rooibos tea wastes	7.20	70.29	3.74	45.4	5.7	2.9	46.0	< 1	10–17	0.007	[42, 44, 51]

consist of an aromatic ring a connected to a third ring B by a carbon–carbon bond and an oxygen-containing heterocyclic ring C connected to an aromatic ring A. In the previous studies, as the amount of carrots added to turnip juice increased, the total acidity, dry matter, ash, total phenol, total anthocyanin, colour density and index increased. The distribution of these six anthocyanins commonly found in nature in edible parts of plants is as follows; cyanide (50%), pelargonidine (12%), peonidine (12%), delfinidine (12%), petunidine (7%) and malvidine (7%) [58].

3 Results and discussion

3.1 Effect of pH

The pH in the media is one of the most important parameters that affect both the adsorbent surface and the adsorb type. In the study, other parameters were kept constant to investigate the effect of changing pH values on anthocyanin. The adsorption studies were performed at varying pH values which were 2.03–3.04–5.72–6.37–7.02–8.92–9.37–10.03–11.20. At 20 °C temperature, 2 g adsorb was added and the values and colour distributions of the anthocyanin pH changes in the structure of turnip juice waste water at the end of adsorption lasting 30 min were given in Table 4.

Table 4 shows that an important feature of anthocyanins is the change in colour intensity and tone depending on pH and even the disappearance of colour. This is due to the chemical complex composition of the molecule of anthocyanin in aqueous environments and its self-rearrangement. In this case, depending on the pH value, different colours or colourless forms emerge. An anthocyanin produces stable colour only at acidic pH values. In acidic aqueous solutions, anthocyanins occur in 4 balance types: quinonoid base, flavilium cation, carbinol or pseudomonas base and shield. The main anthocyanin in purple carrots is a phenol glycosylated cyanide, and there is a change in the blue colour above pH 5.0. In accordance with the analysis and literature, the compounds of anthocyanins act as an indicator based on the pH value of the medium and give different colours in different pH values. Anthocyanins often give red colour at low pH and blue-purple colours at alkaline pH. In light of experimental studies, holding on adsorbent surface is expected to occur according to the pH value of wastewater and adsorb surface condition. In general, adsorption rate increases in pH values where substances are neutral. However, since the wastewater structure contains a lot of hydrogen and hydroxide ions chemically, this situation can be associated with the risk of less coating of adsorb, as adhesion competition between these molecules and adsorb may occur.

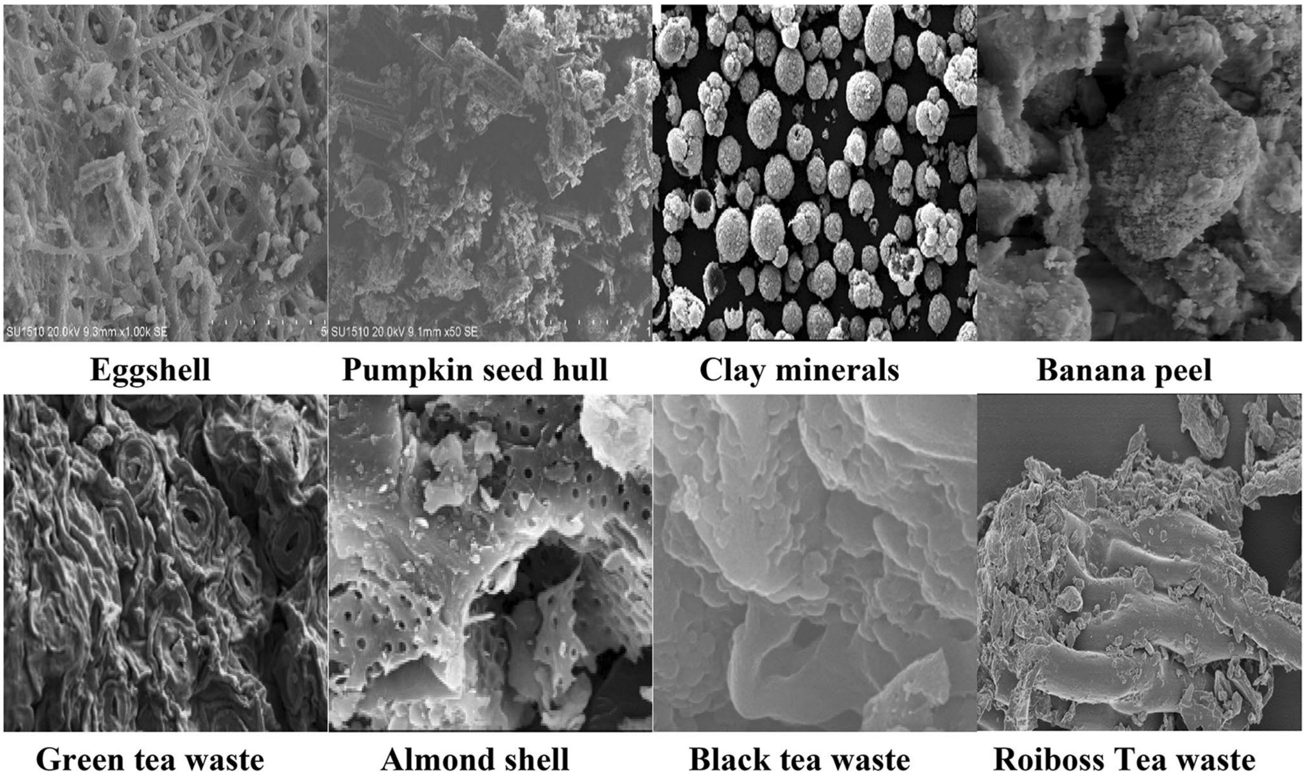

Fig. 4 SEM images of the natural adsorbents

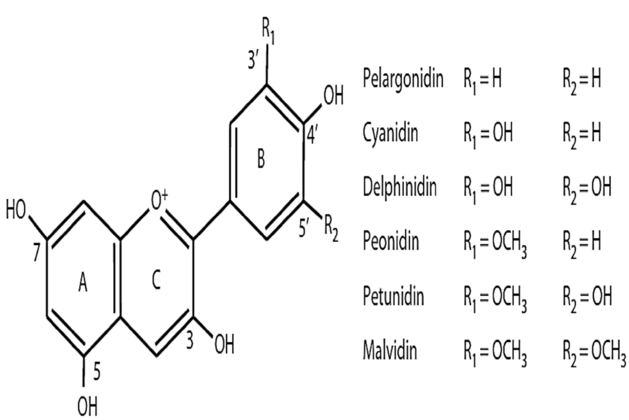

Fig. 5 Structures of anthocyanins commonly found in nature [59, 60]

Table 4 Anthocyanins colour change status at different pH values

pH value	pH balancer	Colour change
2.03	0.01 M HC1	Pink–red
3.04	0.01 M HC1	Pink–red
5.72*	Raw Wastewater	Light purple
6.37	0.01 M NaOH	Purple–pink
7.02	0.01 M NaOH	Light blue
8.92	0.01 M NaOH	Blue
9.37	0.01 M NaOH	Blue–green
10.03	0.01 M NaOH	Blue–green
11.20	0.01 M NaOH	Green

*Initial pH value

3.2 Colour measurements

In experimental studies, colour removal caused by anthocyanin was not provided with adsorbent materials and anthocyanin played an important role in wastewater such as methyl-orange, phenolphthalein (Fig. 6). In the structure of the used adsorbents, especially iron, aluminium, calcium, copper, magnesium etc. it can be said that many ion groups and molecules of anthocyanins can form complex and eventually become a stable blue or violet of red colour and gain a new chemical structure. As mentioned earlier, there is no specific study in the literature on the treatment and color removal of turnip juice wastewater.

In the basic structure of the anthocyanins consisting of 15 carbon A and B, two phenyl rings are connected to each other with a three-carbon structure (Fig. 5). This structure usually forms a second ring and is called the C Ring. In particular, the colour of the anthocyanins is related to the change in the B-ring in chemical structure, and the increase in OH group number of the B-ring

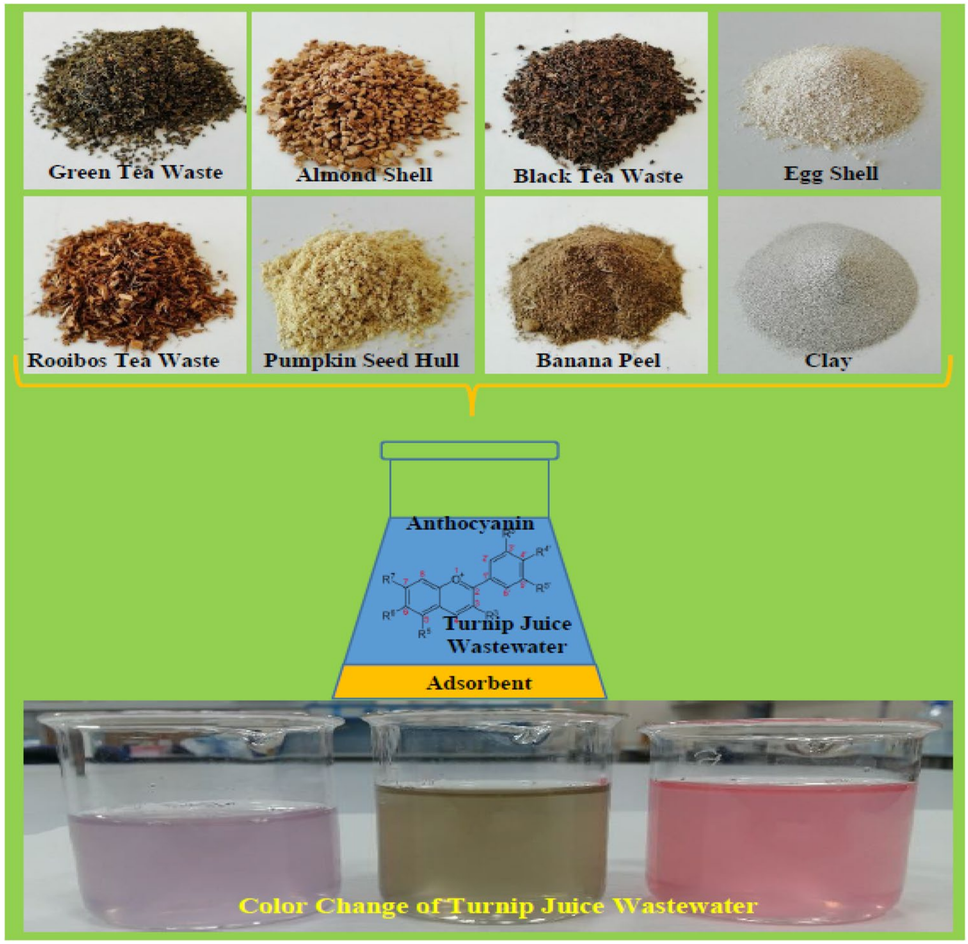

Fig. 6 Anthocyanin colour experiment results with different adsorbents

causes the colour of the anthocyanins to become blue and the increase in the OCH3 group (methoxylation) causes the colour to become red. As the number of OH groups in the molecule increases, the wavelength of the anthocyanin gives maximum absorption is growing and at this point, the colour becomes a blue form. The increase in the number of OCH3 groups makes the colour more red as the group of OH– increases in the chemical form of anthocyanins found in the structure of the wastewater. Because anthocyanins are highly sensitive to pH because of the groups involved in their chemical structure, they are involved in both the literature and the studies of pH because it is effective on the colour, intensity, stability and structure of anthocyanins. Colour analysis of the anthocyanins in the present wastewater was performed with RES method and the results were shown in Table 5 and Fig. 6. RES values were calculated by taking the average of 3 repeated studies in the wavelengths of 436–620 nm range. As a result of pH and colour tests, the colour removal caused by anthocyanin could not be obtained with adsorbing materials and the anthocyanin acts as a pH indicator in wastewater such as methyl-orange, phenolphthalein. It can be said that hydroxyl groups and methoxyl groups have a significant effect on the colour of anthocyanins.

Table 5 Calculated RES values of raw wastewater and adsorbents

RES value (1/m)	Average values	Removal efficiencies (%)
Raw wastewater	14.63	0
Almond shell	13.13	10.25
Eggshell	14.43	1.37
Pumpkin seeds	14.00	4.31
Banana peel	12.57	14.08
Rooibos tea waste	14.00	4.31
Green tea waste	13.60	7.04
Black tea waste	13.30	9.10

4 Conclusion

In the study, adsorption experiments were performed with different adsorbents for the removal of the anthocyanin compounds that gave colour to the turnip water wastewater. Especially the anthocyanins are very unstable and very sensitive to deterioration. Stability and colour status of anthocyanins; the presence of oxygen, light density, pH, hydrogen peroxide, ascorbic acid, enzyme (polyphenol oxidase and peroxidase), chemical structure, co-pigment presence, metal ion, sugars and decomposition products are affected by various physical and chemical factors.

Due to these factors and the complex structure of anthocyanin the colour removal efficiencies obtained as a result of experiments using natural adsorbents vary between 1% and 14%. In the study, the highest colour removal efficiencies were obtained in banana peel, almond peel and black tea waste 14.08%, 10.25%, 9.10%, respectively.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no confict of interest.

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