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Extraction of anthocyanin from plum peels

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ABSTRACT

Food colors are added to food to give attractiveness or compensate for the natural color loss due to light, air, moisture, etc., improving the safety of food products, and enhancing beneficial health effects is a necessity and a major challenge. Anthocyanins are the water-soluble pigments in nature. Anthocyanin extracted from plum by using various methods such as solvent extraction method, enzyme assisted method, and ultrasonic-assisted extraction method, aqueous to phase extraction method, subcritical water extraction method, and microwave-assisted extraction method. The commonly used method is solvent extraction. Especially for cyanidin and peonidin-3-glucoside. Identification of anthocyanin can be done by various chromatographic methods, which include thin-layer chromatography high-speed chromatography, reversed-phase ion-pair chromatography. In the present study, HPLC is used for the qualification of anthocyanin content.

***Keywords*:** *Anthocyanin, Natural Colorant, Solvent Extraction, HPLC*

1. INTRODUCTION

Food colors are added to different types in order to increase their visual attractiveness or to compensate for natural color variations, to make up for color loses following exposure to light, air, moisture and variation in temperature. Anthocyanins are widely used in the food industry as an alternative of synthetic colorants (M.T.M. Assous, M.M. Abdel-Hady, et al., 2014). A plum is a sweet, juicy and succulent fruit. It is about the size of measuring about **5-6 cm** in diameter and weight about **50-70g.**Its scientific names is **Prunes**. When dried, it is called a prune. The plum is a drupe fruit which belongs to the subgenus Prunus (Family Rosaceae) (Ezinne Igwe Karen Charlto,et al.,2016). European plum Prunus Domestica L. is a polymorphic allopolyploid commercially grown worldwide for a variety of uses including fresh fruit, prunes, distilling, and as processed additive ingredients (Stela Dimkova, Darina Ivanova, et al., 2018). The color “plum" takes its name from the fruit. Plum colors can be yellow, red, green or even white. The fruit has a long deep line running down one side, and a smooth stone (seed). The flesh of the fruit is **brown in color and** it tastes very **juicy**. The skin is also edible. Other products that can be made from this jam and wine. It is similar to the apricot. Anthocyanin pigments are more abundant in the peel but they are mainly responsible for the surface color of the fruit (Guo-Ling Liu , Hong-Hui Guo and Yuan-Ming Sun ,2012).

Plums have abundance of bioactive compounds such as phenolic acids, anthocyanins, carotenoids, minerals and pectin(Tetyana Zhebentyayeva , Vijay Shankar,et al.,2019).Anthocyanins represent a group of widespread natural phenolic compounds in plants, and are responsible for their colors(Uros Miljić, Vladimir Puškaš,et al.,2017) These fruits are becoming an increasing popular object of nutritional studies conducted on humans and animals, assessing the effect of plum consumption on the functioning of the organism(preeti birwal et al., 2017). The chemical composition of fresh plums determines to a great extent their taste and technological qualities (Stela Dimkova, Darina Ivanova, et al., 2018). For many decades plums have been used in Indian medicine as a component of natural drug (Preeti Birwals.et al.,2017) Whole fruit anthocyanin content of up to about 30 mg per 100 g. (Joanna milala,Monika kamala,et al.,2013)Plums have low calorie content and relatively high nutritive value. Anthocyanin content in fruit peel is from 1.93 to 19.86 g/kg peel(Preeti Birwals.et al.,2017).(k. Sherpa , s. k. mahato,et al.,2014)They contain carbohydrates, first of all sucrose, glucose and fructose, organic acids, e.g., citric and malic acids, fiber (pectin’s), tannins, aromatic substances and enzymes.

Plum is the type of (Prunus.Domestica.L) packed with high level of nutrients and mainly cultivated in Europe, Asia. It is consumed raw and also in dried form. Plum fruit is suitable for the all type of extraction. (k. Sherpa , s. k. mahato,et al.,2014).It contains of vitamins, minerals, fiber, and enzymes that are good for digestive system (k. Sherpa , s. k. mahato,et al.,2014).plum have abundance bioactive active compounds such as phenolic acids, anthocyanin, carotenoids, minerals and pectin(Stela Dimkova, Darina Ivanova ,2018).

2. MATERIAL AND METHODS

2.1 Sample

2.1.1 Plum: Plum is the type of Prunus. Domestica packed with high level of nutrients and mainly cultivated in Europe, Asia. Plum colors can be yellow, red, green or even white. The flesh of the fruit is brown in color and is tastes very juicy. The skin is also edible. Plum fruit contains both carotenoids and anthocyanin (George Manganaris, Ariel Vicente,2008) . Other products that can be made from this jams and wine. It is similar to the apricot. Plums are a rich source of polyphenols, there are most of all flavonols, and caffeic acid derivatives: non chlorogenic acid, chlorogenic acid, cryptochlorogenic acids well as anthocyanins and flavonols (Guo-Ling Liu, Hong-Hui Guo and Yuan-Ming Sun,2012).It is closely related to the apricot. Flesh is juicy and varies in color from yellow to red(Dorota Walkowiak-Tomczak2008). Plums are a raw material rich in phenolic compounds, which is correlated with its antioxidant capacity (Dorota Walkowiak-Tomczak2008). Pigments are more abundant in the peel but anthocyanins are responsible for surface color of the fruit. Red color of the plum skin is due to anthocyanins, pigment is soluble in water, so plum fruit is suitable for the all type of extraction (k.sherpa et al., 2014). Plum have abundance bioactive active compounds such as phenolic acids, anthocyanin, carotenoids, and minerals and pectin.

2.1.2 Chemicals: The extraction of anthocyanin was done using solvents such as methanol, ethanol, water acidified with acetic acid and ethanol in hydrochloric acid medium (Alessandra Cristina Pedro et al., 2016)

2.2 Extraction of anthocyanin

The fruits were cleaned and washed thoroughly under tap water,(Granato ,D,calado, V.M .,A & Jarvis , B .2014). Then the extraction of anthocyanin was done by using solvents such as methanol, ethanol, citric acid, water acidified with acetic acid and ethanol in hydrochloric acid medium. Among these solvents ethanol and citric acid are less toxic so the anthocyanin was extracted by solvent extraction by using chemicals like ethanol and citric acid 1.0 mol/L.

Weighed 10g of the ground black rice and it was mixed with ethanol and citric acid in the ratio 80:20. The extraction was performed by using the magnetic stirrer for constant stirring and its was protected from light. To standardize the value of extraction was done at different time 30, 50, 80 mins and temperature 25, 40, 60℃.After processing the extract was filtered by using whatman #1 paper. The extract was stored at 20℃ in the polyethylene bottles, protected from light.

2.3 Quantification of total anthocyanin content:

The total anthocyanins were extracted from plum fruit skins were done by:

2.3.1 Solvent extraction: For extraction of anthocyanins the most commonly used solvents is methanol, ethanol, water acidified with acetic acid and ethanol in hydrochloric acid medium (Francis, F .J. (1989). Ethanol and organic acids are desirable because they are less toxic than methanol and hydrochloric acid (Escarbino –bailon, m.t., Santos- buegla,c.,& rivas –gonzalo, j.c.,2004). Anthocyanins are the principal water-soluble pigments responsible for the red, blue, and purple colors (Granato, D, calado, V.M., A & Jarvis, B, 2014). The combination of these factors and the determination of optimal conditions are important in order to obtain a suitable yield of anthocyanins (Alessandra Cristina Pedro et al., 2016). From a practical standpoint, research using random extraction time, concentrations of solvents, and temperatures has been conducted to extract anthocyanins and other phenolic compounds from plum(Alessandra Cristina Pedro, , Daniel Granato, Neiva Deliberali Rosso ,2016).Response surface methodology (RSM) is a method used to develop and optimize processes and products (Granato ,D,calado, V.M .,A & Jarvis , B ,2014). and it can be used to determine the best conditions for the extraction of chemical compounds from natural products(Granato ,D,calado, V.M .,A & Jarvis , B ,2014).

The values for temperature were 10, 30 and 50 ℃, while an extraction time of 20, 50 and 80 min and solid–liquid ratios of 1:15, 1:30, and 1:45 were studied. One gram of finely ground black rice was added to a mixture of ethanol and citric acid 1.0 mol per L in the ratio 80:20(Alessandra cristina pedro et al.,2016). The extraction was performed in a thermo stated cell, protected from light, under constant stirring (laercio galvao maciel et al., 2018). The extraction time, temperature and solid–liquid ratio were determined according to the experimental design (Alessandra Cristina Pedro,, Daniel Granato, Neiva Deliberali Rosso ,2016). After all the processing steps , they were filtered (qualitative Whatman #1 paper) using the solvent of extraction the residue and filter paper were rinsed completely The extract was stored at 20 ℃ in polyethylene bottles, protected from light.

3. RESULTS AND DISCUSSION

Extraction of anthocyanin (solvent extraction method): The anthocyanin pigments were obtained by using solvents of ethanol and citric acid 1.0 mol/L in the ratio of 80: 20, which is done in magnetic stirrer with hot plate equipment at constant time and temperature. The milled black rice sample used for each extraction was about 10g with the proportion of 100ml of solvents in the ratio of 80:20. The extraction of anthocyanin is done by the variations of time and temperature as follows:

Table 1: Temperature and Time Conditions

Temperature (℃)	Time (Mins)
30	30
	50
	80
40	30
	50
	80
70	30
	50
	80

These values of different time and temperature were established from the earlier experiments and studies. The sample extracted was filtered by Whatmam filter paper No#1. The filtered sample was about 50-60 ml for each extraction. The total extracted sample was 320 ml, which was concentrated to remove the solvents, and the 10 ml of remaining precipitate is the anthocyanin pigments.

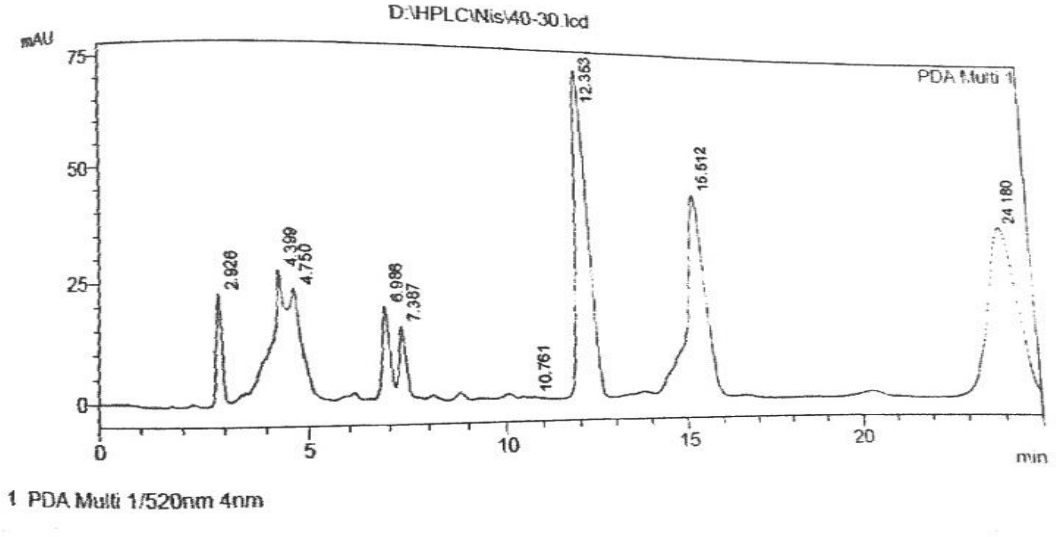

Fig. 1: HPLC chromatogram of anthocyanin pigment at 40 ℃- 30 mins. Wavelength (nm):520nm

Table 2: HPLC analysis for 40℃ at 30 mins

S no.	Ret. Time	Anthocyanin	Peak area of standard curve	Peak area of anthocyanin	Height (%)	Percentage (%)
1	2.926	DELPHINIDIN	21.45	2.96	8.648	0.063
2	4.399	CYANIDIN	59.214	8.241	10.018	0.488
3	6.986	PETUIDIN	274.65	3.558	7.404	0.977
4	12.353	PELARGONDIN	121.62	24.195	27.703	2.943
5	15.512	PEONIDIN	174.02	23.282	17.202	4.052
6	24.180	MALVIDIN	24.62	28.506	14.543	0.702

The extracted anthocyanin pigment was about 92.2% per 100grams of plum. The HPLC chromatogram reveals the highest peak is petunidin component was about 40.52%. This result was compared with the research paper it shows that the percentage of anthocyanin pigment is 97.3% per 100 grams of plum.

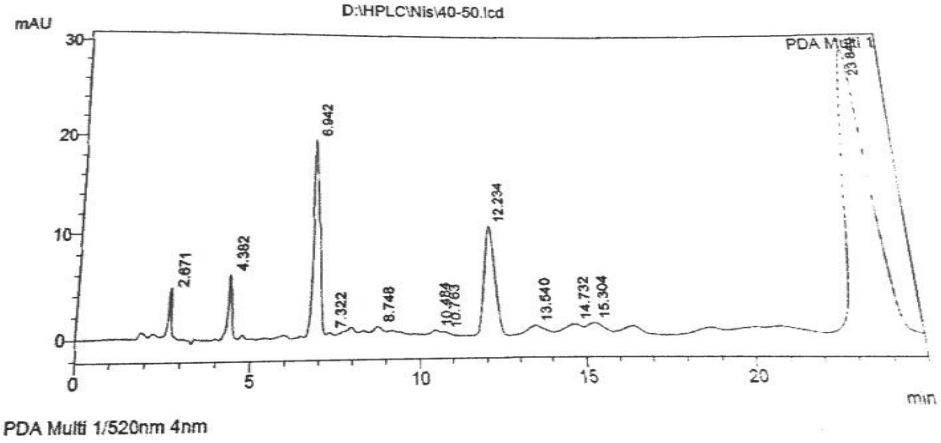

Fig. 2: HPLC chromatogram of anthocyanin pigment at 40 ℃- 50 mins: Wavelength (nm): 520nm

Table 3: HPLC analysis for 40℃ at 50 mins

S no.	Ret. Time	Anthocyanin	Peak area of anthocyanin	Peak area of anthocyanin	Height (%)	Percentage (%)
1.	2.671	DELPHINIDIN	21.45	1.205	6.334	0.026
2.	4.382	CYANIDIN	59.214	2.229	8.230	0.136
3.	6.942	PETUNIDIN	274.65	11.504	26.386	3.160
4.	12.234	PELARGONIDIN	121.62	11.275	14.414	1.371
5.	15.304	PEONIDIN	174.02	1.326	1.217	0.231
6.	23.840	MALVIDIN	24.62	68.9	39.267	1.696

The extracted anthocyanin pigment was about 66.2% per 100grams of plum. The HPLC chromatogram reveals the highest peak is petunidin component was about 31.60% and the least peak is malvidin component of about 16.96%. This result was compared with the research paper it shows that the percentage of anthocyanin pigment is 90.3% per 100 grams of plum.

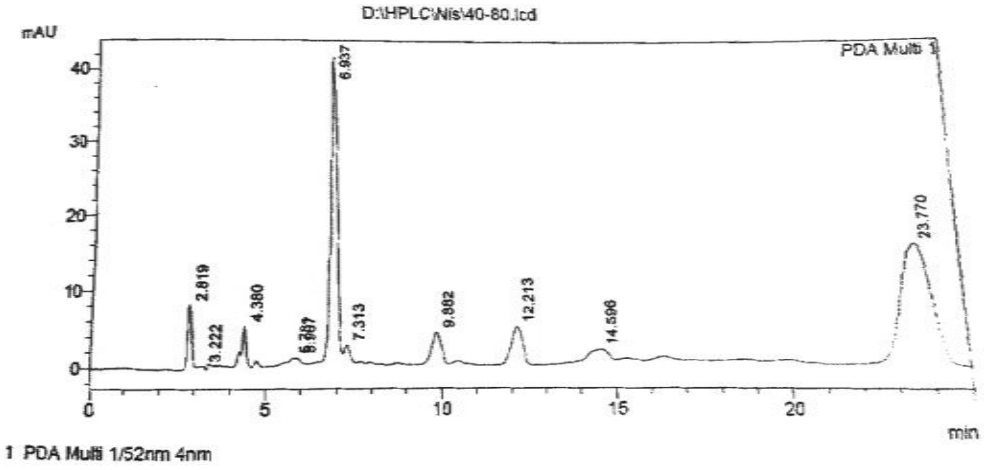

Fig. 3: HPLC chromatogram of anthocyanin pigment at 40 ℃- 80 mins Wavelength (nm):520nm

Table 4: HPLC analysis for 40℃ at 80 mins

S no.	Ret. Time	Anthocyanin	Peak area of anthocyanin	Peak area of anthocyanin	Height (%)	Percentage (%)
1.	2.819	DELPHINIDIN	21.45	3.998	9.982	0.086
2.	4.380	CYANIDIN	59.214	1.369	5.050	0.081
3.	6.937	PETUNIDIN	274.65	28.787	49.075	7.906
4.	12.213	PELARGONIDIN	121.62	5.966	5.796	0.726
5.	14.596	PEONIDIN	174.02	2.919	1.702	0.508
6.	23.770	MALVIDIN	24.62	50.683	18.830	1.248

The extracted anthocyanin pigment was about 105.5% per 100grams of plum. The HPLC chromatogram reveals the highest peak is petunidin component was about 79.06% and the least peak is Maldivin component of about 16.96%. This result was compared with the research paper it shows that the percentage of anthocyanin pigment is 103.6% per 100 grams of plum.

4. CONCLUSION

The optimization of anthocyanin pigment was evaluated by the effect of temperature and time on the extraction of compound from plum. The temperature for extraction is 40℃ for 80 mins had proved to be the best condition to maximize the total content of anthocyanin; this extract can be used by the food industry as natural food colorant. The extraction was done by solvent extraction method with different time and temperature. The anthocyanin pigment was detected by HPLC analysis. The best temperature and time of 40℃ for 80 mins. The extracted anthocyanin pigment (105.5mg of plum) presented a pleasant reddish color.

For further research and studies the extracted anthocyanin can be incorporated, fortified and can be used as food additives. It can be incorporated in yogurt and cream.cyanindin-3-glucoside was the main anthocyanin identified in plum. In a possible industrial application of the extract, the stability parameter must be taken into an account for better preservation of the sensory aspect of the product.

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