(August Special Issue)

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Estd. 1949

Journal of
The Maharaja Sayajirao University of Baroda

Certificate of Publication

Certificate of publication for the article titled:

STUDIES ON EXTRACTION OF FROM EGGPLANT PEEL AND ITS APPLICATION IN VALUE-ADDED PRODUCT

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Volume No . 56 No. 8 -2022
in
Journal of The Maharaja Sayajirao University of Baroda

ISSN : 0025-0422

(UGC CARE Group I Journal)

Editor
Journal MSU of Baroda

STUDIES ON EXTRACTION OF FROM EGGPLANT PEEL AND ITS APPLICATION IN VALUE-ADDED PRODUCT.

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ABSTRACT

Fruits and vegetables are important source of various nutrients like vitamins, minerals, bioactive compounds, etc. Natural colour pigments are gaining importance worldwide due to their potential health benefits and attractive colours. Anthocyanin, a bioactive compound is extracted by different methods, but in this work conventional solvent extraction is considered as the most suitable/potential method for eggplant. Major anthocyanin found in eggplant is nasunin. Purification of the crude extract is done by using macroporous resins Amberlite XAD4 employing adsorption and desorption. This natural pigment from eggplant peel can be incorporated into varieties of food products and development by using eggplant flesh flour which increases its nutritive value, attractiveness and acceptance towards consumers. This work mainly emphasizes on extraction method of anthocyanin, sits purification and development of new product with incorporation of colour in it.

Keywords: *Anthocyanin, Extraction, Purification, Stability, Product Development*

INTRODUCTION

Colour in one form or another is added in our food for centuries. It is known that Egyptians coloured candy and wine was coloured as long ago as 400 BC. The earliest record for the use of natural dyes was found in China, dated 2600 BC. The art of making coloured candy is depicted in paintings on Egyptian tombs as far back as 1500 BC. It was the discovery of the first synthetic dye, mauve, in 1856, by Sir William Henry Perkins in England that prompted a search for other dyes. At the turn of the century there were nearly 700 synthetic colorants available and very little control was exercised over the type and purity of the colorants offered for use in foods. This led to colours being added for decorative purposes and unfortunately to disguise low-quality foods. An established list of permitted synthetic food colours eventually came into force in most countries early in this century. Synthetic colours can destroy the nutrients in the food because of their chemical composition and increase hyperactive behaviour in some children (like sodium benzoate, red food dye and various yellow food dyes). Human studies indicated that food colours, (even natural or synthetic) can induce wide range of allergic reactions only in sensitive or atoptic individual (Rajapaksha*etal.*, 2015).

Fruits and Vegetables are naturally low in fat, calories and are cholesterol free, also they are the important sources of various nutrients like vitamins, minerals, fiber and bioactive compounds.The main food biocolorants are carotenoids, flavonoids, anthocyanidins, cholorophyll, betalain, etc Anthocyanin (in Greek anthos means flower, and kyanos means blue) are found in plants (flowers, fruits and vegetables) and are responsible for vibrant colours like most shiny orange, pink, red, violet and blue hues (Jiang *et al*., 2018). Anthocyanins are responsible for the anti-oxidant, anti-inflammatory, anti-cancer properties, etc. These are water-soluble compounds occurring in the cell

sap. All anthocyanins are derivatives of the basic flavylium cation structure. Among the various vegetables, eggplant (Aubergine) is a rich source of bioactive compound anthocyanin, belonging to family solanaceae and they are located mainly in the southeast of Asia (Ferarsa*et al*., 2018). As the pigment is beneficial for human health, they are also incorporated into food products such as confectionary, beverages, jams, jellies, macaroni products for which conventional and modern extraction methods are used. At same scale, conventional techniques like solvent extraction, grinding process are time consuming, less yield and more solvent is used. But in recent times, modern methods are used such as ultrasound assisted extraction, microwave assisted extraction, heat assisted extraction, pressurized liquid extraction, supercritical fluid extraction, pulsed electric field, etc which gives high yield, high quality product, less energy and solvent consumption. The crude extract is concentrated in a rotary evaporator and is thus purified using adsorbents. Purification process is done by using various macroporous adsorbent resins like XAD 7HP, XAD-4, XAD-16, AB-8 resins, etc in a glass column.

Macaroni products, also termed as pasta products, include macaroni, spaghetti, vermicelli, noodles and other similar products, are widely used food for consumption. So due to the high demand of consumption of macaroni products by children and adults, Macaroni product can be developed which is nutrition rich and containing natural anthocyanin pigment in it. Eggplant flesh powder also adds value to macaroni products, as they are rich fibre, magnesium, potassium, etc. They have various nutritional benefits.The main aim of present work is to focus on extraction and purification of anthocyanin from eggplant peel and its application in nutrition rich food product.

MATERIALS AND METHODS

MATERIAL

All the experiments were carried out in G.S. Mandal's Maharashtra Institute of Technology-Aurangabad, Mahatma Gandhi Mission College of Food Technology, Gandheli-Aurangabad, Dr. Babasaheb Ambedkar Marathwada University, Department of chemical technology-Aurangabad

3.1 RAW MATERIALS:

Purple coloured Eggplant (*Solanum Melongena L*.) was purchased from the local market of Aurangabad. The analysis was done in the department of Agricultural Engineering in Food Analysis Laboratory and Centre for Analytical Research Studies (MIT-CARS) and other raw materials necessary for product development like semolina, wheat flour, vegetable oil was also purchased from the local market of Aurangabad.

3.2 CHEMICALS :

All the chemicals were used for experimental analysis of analytical grade like Ethanol (99.9%), HCL (36-37%) (hydrochloric acid), Amberlite XAD-4 resins, etc.

3.3 PROCESSING AND ANALYTICAL EQUIPMENT'S

The following equipment's were used for processing and analysis and were present in food analysis laboratory-MIT College, Department of food chemistry and nutrition-MGM college:

1. ICP-OES
2. Hunter Lab colorimeter
3. Preparative column (Chromatography)
4. Flame Photometer

5. UV-Vis Spectrophotometer

METHOD

3.4 PRE-TREATMENT OF RESINS

Resins were suspended in water to expand the beads before packing in a glass column (I.D.*L: 22*350 mm). Ethanol (140 mL, 95%) was used to wash the column with flow rate of 400 mL/h. Column was washed with water until the eluent was clear. The resin was then eluted with 140 mL of 4% HCL followed by distilled water until pH of the eluent became neutral. The column was then washed with 140 mL of 5 % NaOH, followed by distilled water until the eluent reached a pH of 7.0. Samples of the pre-treated resins were weighed into aluminium dishes and kept in an oven at 60˚C for 24 h (Buran *et al*., 2014).

3.5 EXTRACTION OF ANTHOCYANIN

About 100 g of the peel was blended with 100 ml ethanolic HCL in a blender at full speed. Further, the solution was transferred to a 500-ml glass-stoppered bottle using approximately 50 ml of ethanolic HCL for washing the blender jar. After storing it overnight in a refrigerator at 4˚ C, samples were filtered through whatman No.1 paper using a Buchner funnel. Residue on the filter paper was repeatedly washed with ethanolic HCL until approximately 450 ml of extract collected. Further, it was transferred to 500-ml volumetric flask and store for further use (Ranganna, 1986).

3.6 STATIC ADSORPTION/DESORPTION TESTING.

Pre-treated hydrated resin (1 g) and 25 mL of eggplant peel extract were added to 250 mL Erlenmeyer flasks with stoppers. Flasks were kept for shaking in a water bath shaker at a rate of 45 rpm. Adsorption was conducted at room temperature (25˚C) for 24 h. For static desorption testing, the phytochemical-laden resins were washed with 25 mL distilled water. After water wash, 50 mL of 95% ethanol was added in the flasks. The flasks were kept in the water bath shaker at 45 rpm for 24 h at room temperature (Buran *et al*., 2014). Adsorption and desorption ratios and capacities were calculated using the following equations:

Adsorption ratio

$A(\%) = (Co - Ce) \div Co$

Adsorption capacity

$qe = Co\text{-}Ce \times (Vi) \div (1 - M)W$

where,

A= adsorption ratio(%);

qe= adsorption capacity(mg/g dry resin)at equilibrium;

Co and Ce= initial and equilibrium concentrations of anthocyanins or total phenolics in the eggplant peel extracts (mg/L);

M= moisture content of the resin (w/w%);

W= initial weight of the resin being used (g);

Vi= volume (mL) of the extract used.

Desorption ratio

$D\% = Cd\frac{Vd}{(Co-Ce)Vi} \times 100$

Desorption Capacity

$qd = Cd \times \frac{Vd}{(1-M)W}$

% Recovery

$R = \frac{CdVd}{CoVo} \times 100\%$

where,

D=desorption ratio (%);

qd =desorption capacity (mg/g dry resin);

R= recovery (%) after desorption is completed;

Cd=concentration of anthocyanins in desorption solution (mg/L);

Vd = volume of the desorption solution (mL)

Dynamic adsorption/desorption testing

A glass column with a fritted disk (I.D.$\times$ L: $19 \times 400\ mm$) was loaded with XAD 4 resin with a resin bed volume of 30 mL. Eggplant peel extract was loaded into this column using a flow rate of 2, 5 or 10 BV/h, respectively. The adsorbate-laden column was then washed with water (150 mL) to remove sugars and other compounds that did not adsorb on the resin. Phytochemicals were desorbed using 95% ethanol at a flow rate 2, 4 or 6 BV/h, respectively. Eluent was collected and analyzed for total anthocyanin.

3.7 PREPARATION OF EGGPLANT FLOUR

The purchased eggplants were washed under running tap water properly and the peeled off using a sharp knife. The inner portion was cut into slices and then treated with 5% salt solution for 15 minutes to avoid browning. The slices were then kept into cleaned and sanitized trays for drying in the tray/cabinet dryer at 70° C for 24 hours. The dried eggplants were grinded into a kitchen blender till fine powder and were further sieved.

3.8 FORMULATION OF MACARONI PRODUCT

The product was made using eggplant flesh flour, semolina flour and wheat flour as the ingredients. Different formulations were made of product using wheat flour in less amount to make the product less gluten content. The macaroni was made using conventional technique by cutting the dough with knife and drying was carried in ambient air for 5-6 hours.

3.9 ANALYSIS

3.9.1 Determination of total anthocyanin content.

The total anthocyanin content was determined by the method mentioned in Ranganna, 1986. A UV-VIS Spectrophotometer was used for spectral measurements at 525nm. The anthocyanin content was calculated based on cyanindin-3-glucoside which have molecular weight of 449.1 and extinction coefficient of 29,600. 100g of berry was macerated, filtered, washed and made to 500 ml as given under extraction. Different standards were made like 1ml, 2ml, 3ml, 4ml and 5 ml and the volume were made till 25 ml using ethanolic HCL for being into the linear region of Lambert-Beer law.

$$\text{Total OD/100 g of berry} = \frac{\text{Absorbence at 525 nm} \times \text{volume made up of the extracts used for colour measurement} \times \text{total volume of sample}}{\text{ml of the extract used} \times \text{weight of sample taken}} \times 100$$

$$\text{Total Anthocyanin content} = \frac{\text{Total OD}}{\text{E value}}$$

3.10 ANALYSIS OF ANTHOCYANIN EXTRACT

3.10.1 Determination of pH, total solids

The measurement of pH of anthocyanin extracts before and after adsorption was carried out using a pH meter (Elico). Soluble solid content expressed as refractive index, was measured with hand refractometer (Atago).

3.10.2 Colour Analysis

The colour characteristics (Hunter CIE L*, a* and b*) of anthocyanin before and after purification was measured using a colorimeter (ColorFlex EZ). The sample was placed in 1 cm optical glass cell and CIE L*, a* and b* values were measured in the transmission mode.

3.11 PHYSICOCHEMICAL ANALYSIS OF EGGPLANT FLESH FLOUR/POWDER

3.11.1 Moisture Content

Moisture content of the eggplant flesh powder was determined using the hot air oven method (AOAC, 2000). For determination of moisture content, 5 gm of sample was placed in hot air oven at 105 ºC for 3 hours and moisture content is calculated as:

3.11.2 Protein Content

Crude protein was estimated using the micro Kjeldahl method (Pelican Equipments) following steps like digestion at 400 ºC, distillation with boric acid (4%) and then titration against 0.1 N HCL. Protein (%) content was calculated using Conversion factor 5.7 as :

3.11.3 Fat Content

Fat content was estimated using soxhoplus (Pelican equipments). About 1 or 2 gm sample was placed in cellulose thimble using 80/90 ml hexane in the beaker. It is calculated by the formula:

3.11.4 Crude fiber content

Crude fiber was estimated using fibroplus (Pelican Equipments) giving 1.25% sulphuric acid and 1.25% sodium hydroxide (Acid - Base) wash followed by hot air oven at 130ºC and muffle furnace at 550ºC for 2 hours. It is calculated using formula:

3.11.5 Carbohydrate content

Carbohydrates are calculated on the basis of determination of the remaining four parameters. There is a theoretical formula as follows

3.11.6 Ash content

The ash fraction contains all the mineral elements but it allows to nitrogen-free-extract(by difference) from dry matter. Ash content was determined using muffle furnace. 5 gm of sample was previously weighed in silica crucible and kept in muffle furnace at 550ºC for 5 hours. The results were calculated using the formula

3.11.7 Potassium, sodium and magnesium content

Inductively coupled plasma optical emission spectrometry (ICP-OES) (Parkins) was used for the detection of minerals and heavy metals like calcium, iron, Iodine, zinc, magnesium, fluoride, manganese, chromium, copper, cadmium, nickel, etc. These trace metals occurred in minor quality of ppb/ppm (parts per billion/million). Potassium and sodium were determined using Flame Photometer (Elico CL 378). Samples (1 gm) were diluted in 0.12 M ammonium oxalate, Shaked and filtered.

3.12 SENSORY EVALUATION BY HEDONIC SCALE

The sensory characteristics of macaroni were determined using a taste panel consisting of members from Department of Agricultural Engineering, Maharashtra Institute of Technology, Aurangabad. The sensory characteristics of the products were evaluated by using nine-point hedonic scale. A nine-point hedonic scale was used for sensory evaluation of macaroni.

3.13 STATISTICAL ANALYSIS

All analytical tests and experimental analyses were carried out in triplicates and expressed as mean values, coefficient of variance (CV) and standard deviation.

RESULT AND DISCUSSION

Present chapter discusses the results obtained for extraction and purification of anthocyanin pigment from eggplant peels. Also, it discusses about product formulation from eggplant flesh and its nutritional aspects.

4.1 TOTAL ANTHOCYANIN CONTENT IN EGGPLANT PEEL

The anthocyanin extracted from eggplant peel using 95% ethanol 1.5 N HCL (85:15) ethanolic extract was determined by the method of Ranganna, 1986.

Fig No. 4.1 Anthocyanin Extract

Fig No. 4.2 Pigment Extracted Peel

This method was used due to use of low temperature, easily done and low investment at laboratory scale. The pH of anthocyanin was noted as 1.68 and it was dark red in colour **(Fig. 4.1 and 4.2)**. The yield of anthocyanin obtained was 76.42±2.22 mg/100 g of peel.

4.2 PURIFICATION OF ANTHOCYANIN USING ADSORPTION AND DESORPTION

4.2.1 Static adsorption and desorption behaviors of anthocyanin on resin

XAD4 is a non-ionic acrylic ester resin with moderate polarity (hydrophobic in nature). XAD4 has the adsorption capacity and ratio are 1.03 mg/mL and 60%, and has the highest surface area of 750 m^2/g. XAD4 has got little low adsorption capacity by its very small pore envelop size of 100 Å and particle size of 0.3-1.2 mm. Pore size and surface area are determining factors in predicting the adsorption capacity. In desorption tests the, the desorption capacity and ratio is 0.93 and 78.5% with the recovery of anthocyanin on resin is 75.5%. Ethanol concentration above 40% (v/v) could effectively elute anthocyanin from the adsorbent. Hence high concentration (>40 % v/v) of ethanol was used (Jampani*et al*., 2014).

4.2.2 Dynamic adsorption and desorption on XAD4 resin

The best adsorption was observed at lowest flow rate of 1 mL/min. Less flow rate allows more time for the adsorbent molecules to interact with the active sites of the adsorbent. In contrast, high flow rate

requires less time but has a negative impact on the adsorption capacity since the residence time of anthocyanin is less. Hence the breakthrough point is reached quickly. In general, the eluent concentration on reaching the 5% of the inlet concentration is defined as the breakthrough point. For dynamic adsorption 6.5 bed volume (BV) of solution and flow rate of 1 mL/min are required as standard conditions. Dynamic desorption is based on the volume of desorption solution and the concentration of solute therein. Acidified aqueous ethanol is used for elution of anthocyanin by maintaining the flow rate at 1 and 2 mL/min. 4 BV desorption solution elute the anthocyanin at flow rate of 1 mL/min and 6 BV has 2 mL/min flow rate.

4.3 EVALUATION OF ANTHOCYANIN SOLUTION

Besides anthocyanins, eggplant extract has many more components, where few of them are analyzed. Hence, the evaluation was carried out with respect to total soluble solid (TSS), pH of the anthocyanin before and after purification with XAD4 resins. Besides, the hunter lab L_,a_,b_, values were also noted for crude extract- 21.13, 30.06, 22.77 and Purified extract-24.56, 36.89, 28.09. The results shown where the pH decreased from 1.68 to 1.4 after purification, the concentration of solids for crude extract was 19 and 14 after purification.

4.4 PREPARATION OF EGGPLANT FLESH FLOUR

4.4.1 Salt Treatment

The eggplant was sliced and given salt and lemon treatment to avoid browning of the slices. The treatment where the analysis of the browning effects was done using sensory testing like taste, appearance and texture. Various treatments were given to 20 g of sample each and were dipped in salt solution till 15-20 minutes. After the salt treatment, the browning was firstly seen in 1% (1gm salt in 100 ml water and 1% lemon is 1ml lemon squeezed in 100 ml water) sample, then followed by 2% and 3%. Browning was less in the sample of 4%. Further, 5% sample remained stable after removing it from salt solution for a longer time. Therefore, sample 5% was selected for further drying of the slices. The lemon treatment was not considered as suitable due to its taste for further powder preparation of eggplant flesh and browning effect was also seen soon after the treatment **(5%),** so the salt treatment of 5% treatment was selected for the drying purpose which showed minimum browning and a good appearance and taste.

To limit the oxidation phenomenon of eggplant, various treatments are given. Sodium chloride is used for strengthening of cell walls. The cell walls are more stable to different treatments. This prevents the destruction of cell compartments and also the contact of polyphenol oxidase (PPO) with polyphenols in the vacuoles. (Ioannav and Ghoul, 2013)

4.4.2 Drying of eggplant slices

Drying was carried out in a tray dryer at 70ºC for about 12 hours. Due to the salt treatment, the slices did not turn brown. Browning results in loss of eye appeal for consumers and may adversely affect nutritional and sensory properties of fresh cut eggplant. The reason for browning is the level of phenolics and their oxidizing enzymes polyphenol oxidase (PPO) as studied by Mishra *et al.,* 2012.

The results shown in the below **figure 4.4**shows about the effect on drying of eggplant where no browning is seen. During drying, browning does not occur in the slices and the thermal temperature for drying is also suitable**.** Itis the totally dried sample where browning is very less and its taste is also

acceptable for further processing. Therefore, it is noted that the treatments given to eggplant slices are effective for powder preparation.

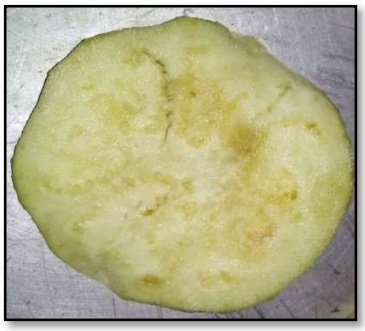

Figure 4.3 Before Drying

Figure 4.4 After Drying

4.4.3 Flour Preparation

The dried samples were collected from the trays and weighed. They were grinded in the grinder (mixer) to form powder. The powder was finely sieved through the cotton cloth for fine sieving and stored in the containers.

4.5 FORMULATION OF MACARONI PRODUCT

The ingredients like eggplant flesh flour, semolina and wheat flour are the rich source of dietary fibre, minerals (potassium, magnesium), protein, etc. Semolina used was also ground into powder/flour for proper mixing. As semolina has low elasticity and high plasticity than most flours, so it is one of the important ingredients used for macaroni preparation. Wheat flour is added in less quantity due to its more content of gluten. Gluten has been tied to bowel diseases, alter gut bacteria and increase intestinal permeability in people with this disease**.** In normal people, the gluten is not good for digestion. Eggplant flour is added to increase the nutritional content of macaroni. Different formulations are made based on the colour and texture of the dough. As eggplant flour is little bitter in taste it is added in less proportions.

Sr. no.	Eggplant Flesh Flour (gm)	Semolina Flour (gm)	Wheat Flour (gm)
M_0	0	80	20
M_1	5	75	20
M_2	10	70	20
M_3	15	65	20

Table 4.1: Different formulations of Macaroni product/100g

These formulations are made on the basis of few trails in making the dough and observing its colour, appearance, texture. wheat flour is kept constant. Values of eggplant flesh flour vary from 5% 10% and 15% as discussed in **table 4.1.** The semolina flour is replaced by certain amount as the eggplant flour values increases. The formulation M_2 (with 10: 70: 20 proportion) was selected based on taste,

colour and texture, and further anthocyanin was added in certain amount in the dough. The purified anthocyanin was added in various concentration in the formulated dough M_2**.(Fig No. 4.5)** The concentrations are 0.5%, 1% and 1.5% and
are studied by*sarabandi et al.* 2019 in gummy candy.

Figure 4.5 M_2 with anthocyanin.

4.6 ANALYSIS

4.6.1 Proximate analysis of raw material for macaroni preparation

Chemical properties were analysed to check the quality of raw materials. The nutritional composition of semolina, eggplant flesh flour and wheat flour

As eggplant flesh flour is added for fortification of product, it is analysed using various instruments to get idea about nutritional contents such as Moisture content, protein content, fat content, fibre content, potassium, magnesium. The eggplant flesh and eggplant peel were also tested. The major nutrient found in eggplant flesh is fibre, magnesium and potassium. The potassium found in eggplant is nearly 235.9 ppm, calcium is 47.197 ppm and magnesium are 51.150 ppm. The proximate analysis of eggplant flesh and eggplant peel was also carried out. The protein content in eggplant flesh flour is increased as compared to raw flesh. Fat content is decreased from 1.8 % to 1.13%.

4.6.2 Organoleptic Evaluation of macaroni

The sensory characteristics of macaroni were determined using a taste panel consisting of members from Department of Agricultural Engineering, Maharashtra Institute of Technology, Aurangabad. The sensory characteristics of the products were evaluated by semi-trained panel using nine-point hedonic scale. A nine-point hedonic scale was used for sensory evaluation of macaroni. The acceptability statements and their marks given in **table 4.2**

Sample	Parameters				
	Colour	Texture	Flavour	Taste	Overall acceptability
M_0	7.6	8.2	6.9	7.5	7.55
M_1	7.7	7.8	7.0	7.8	7.57
M_2	8.0	7.6	7.2	8.0	7.7
M_3	7.2	7.4	6.7	7.1	7.1
Std. Dev.	0.33	0.34	0.20	0.39	0.26
Mean	7.62	7.75	6.95	7.6	7.48
CV%	4.33	4.40	2.99	5.15	3.50

Table 4.2: Sensory evaluation of macaroni

CONCLUSION

The total anthocyanin content was found to be 76.42±2.22 mg/100 g of peel in eggplant. The macaroporous resin XAD4 was preferred suitable for purification.The pH and soluble solids of crude extract was 1.68 and 19 ºB whereas of purified extract was 1.4 and 14 ºB respectively.40% acidified

ethanol was considered to effectively elute anthocyanin from adsorbent. The eggplant flesh slices were given 5% salt treatment and drying in cabinet tray dryer.Eggplant flesh flour was analysed and was found to increase the fibre, potassium, calcium and magnesium content of the formulated product.The organoleptic score of the M_2 sample was better than M_0, M_1 and M_3 samples.

ACKNOWLEDGEMENT

The Author is grateful to the department of Agricultural Engineering, MIT College Aurangabad and also thankful to Guide Dr. D.T Bornare and Co-Guide Dr. Deepak Jadhav for their precious guidance and support.

I also provide thanks to MIT-CARS (Centre for Analytical Research Studies) for their support and opportunity to carry out the research work.

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