Journal of Agricultural Engineering and Food Technology
Print ISSN: 2350-0085; Online ISSN: 2350-0263; Volume 2, Number 1; January-March, 2015 pp. 17-21

http://www.krishisanskriti.org/jaeft.html

An Economical Approach for Production of Purified Ellagitannin Powder from Fresh and Fermented Pomegranate Peel

Satish C. Kushwaha*, M. B. Bera and Pradyuman Kumar

Department of Food Engineering & Technology, Sant Longowal Institute of Engineering and Technology, Longowal, Sangrur, Punjab-148106
E-mail: [1]satish. ch. kush@gmail.com, [2]pradyuman2002@hotmail.com

Abstract—*Pomegranate (Punicagranatum L.) fruit is widely being consumed and its fruit's peel having hydrolysable ellagitannin (ETs: polyphenolic compound) is an underutilized industrial waste from commercial point of view. In this present study an economical and efficient technique has been explored for the production of purified ellagitannin powder from pomegranate peel. Ellagitannin was isolated from fresh and fermented peel, purified with Amberlite XAD-16 resin packed column and converted into ellagitannin powder by vacuum drying. About 38 gpurified ellagitannin powder was obtained from 1 kg fresh pomegranate peel. Total ellagitannin content(TEC) was 89. 78-91. 78 % as GAE possessing antioxidant activity (AOA) of 91. 16-96. 21 % as DPPH. Cost estimation of purified ellagitannin powder preparation process was Rs. 5510 from 1 kg fresh pomegranate peel. Worth of purified ellagitannin powder (38 g) estimated Rs. 25441 in international market. Studied method can be adopted for commercial production of purified ellagitannin powder.*

Keywords: *Pomegranate, Peel, Ellagitannin, Antioxidant activity, Purification*

1. INTRODUCTION

Pomegranate (*Punicagranatum*L.) fruit is widely consumed fresh and in processed form as juice, jam and wine. Pomegranate fruit peel, a byproduct of pomegranate juice industry and rich source of hydrolysable tannin called ellagitannin (ETs: polyphenolic compound) [1]. Different in-vitro and in-vivo studies carried by researchers revealed that ellagitannin extracted from pomegranate peel possess strong antioxidant activity. Pomegranate peel extracts are currently used for treatment of respiratory diseases and in the preparation of tincture, cosmetics and other therapeutic formula [2, 3]. Pomegranate ellagitannin are also being investigated for their potential use as food bio-preservatives and for the formulation of products in the nutraceutical industry [2]. Pomegranate peel attracts attention due to its apparent wound healing properties [4], immune modulatory activity [5], antibacterial activity [6] and antiatherosclerotic and antioxidative capacities [8]. Antioxidative activity has often been associated with a decreased risk of various diseases [12]. In the last few years the identification and development of phenolic compounds or extracts from different plants has become a major area of health and medical related research [6]. Data from literature indicate that 124 different phytochemicals can be found in pomegranate fruit; amongst these phytochemicals, high molecular weight polyphenols (e. g. ellagitannins and the pomegranate-peculiar punicalagin) are likely to mediate the protective effects against a wide range of oxidative and inflammatory disorders, including cancer [10]. Recovery of tannin/ phenolic compounds is commonly performed through a solvent extraction procedure, but at present an ambiguous data on the method and conditions for extraction are available. Sometimes the reports are contradictory, particularly when different raw materials are compared. Sometimes only the total phenol concentration of final extract is reported, but not the total yield. The aim of an extraction process should be maximize the yield of target substances with highest quality/purity[7]. Alcohol as solvent is commonly employed to extract phenolics from natural sources as they give quite high yield of total extract, even though they are not highly selective for phenolics[12, 13]. More than 75% of the panelists accepted the phenolic enriched ice creams in sensory evaluation, which lends supports to such products for commercial introduction to the general public with the potential as functional foods [14]. Patel *et al* [15] investigated the toxicological effect of ellagitannin and concluded that "no observed adverse effect level" (NOAEL) for standardized pomegranate fruit extract was determined as 600 mg/kg body weight/day, the highest dose tested. Li *et al* [16] quantified eight monophenols (including gallic acid, punicalagin, catechin, chlorogenic acid, caffeic acid, epicatechin, rutin, and ellagic acid) in the pomegranate peel to interpret the consistency of the quality test.

Separation, purification and pharmacological effects of ellagitannin have been reported in many papers, but there are very less approaches in respect of economical and efficient process to extract the ellagitannin from fresh as well as

fermented pomegranate peel. In this present study a cost effective and efficient method has been studied to extract ellagitannin from fresh and fermented pomegranate peel.

2. MATERIAL AND METHODS

2.1 Fresh pomegranate fruit

Pomegranate fruits of Baghwa variety were collected from AjadpurMandi, New Delhi. Fruits were washed with distilled water and wiped the surface water with tissue paper. Fruits were peeled manually and separated the peels from aril. Peels including pith and carpellary membrane part of fruit were preserved for ellagitannin extraction.

2.2 Sample Preparation

The obtained fresh pomegranate peels (FPP) were crushed in grinder (Maharaja White lines, India). The ground peel was then passed through 2 mm pore size sieve to ensure uniform particle size of pomegranate peel. The ground peel was stored at -18 ^{o}C in deep freezer till its utilization.

2.3 Chemicals and reagents

Folin Denis ciocalteu reagent, sodium carbonate, methanol (Merck, India), DPPH (CDH, India), gallic acid and XAD-16 resin (Sigma Aldrich, USA) were used in this study.

2.4. Isolation of ellagitannin from fresh pomegranate peel

Hundred gram of grounded fresh pomegranate peel taken in five conical flasks and added 200 ml distilled water in each flask and incubated with shaking in shaker incubator (Kuhner, Switzerland) at 90^{0}C for 18 min. Each flask contentswere centrifuged and supernatant were collected in two liter beaker. Centrifugation process repeated by using copious amount (100ml) distilled water and supernatant collected and combined with whole peel extract.

2.5. Isolation of ellagitannin from fermented pomegranate peel

2. 5. 1. Maintenance of microbial culture and fermentation of peel

Aspergilusniger strain MTCC 281 was procured from Institute of Microbial Technology, Chandigarh. Potato dextrose agar slants were used for the maintenance of *A. niger*. Fully sporulated slants were stored at 4 $^{\circ}$C in refrigerator for further use, followed by sub culturing. Fungal spore inoculum was prepared by adding 2. 5 ml sterilized water containing 0. 1 % tween 80 to a fully sporulated culture. The spores were dislodged by using a sterile inoculum loop under laminar air flow (Labtech, Korea). The number of viable spores in the suspension was determined using the plate count method. 1 ml of the prepared spore suspension containeda concentration of 3. 8 x 10^{9} spores.

2.5.2. Fermentation of peel

About 500 g of fresh pomegranate peel is inoculated with *A. niger*culture (3. 8x10^{9} spore per ml /100 g fresh peel) and incubated at 26 $^{\circ}$C for 7 days in incubator (Macflow Engineering, India). Fermented peel (100 g) taken in 5 conical flask (500 ml) and added 200 ml distilled water in each flask and shaken in shaker incubator at 90 $^{\circ}$C for 18 min. Content of each flask were centrifuged and collected the supernatant. Centrifugation process repeated by using copious amount (100 ml) of distilled water and combined with the whole peel extract.

2.6. Purification of extract by prepared column and drying

AmberliteXAD-16 resin (Sigma Aldrich, USA)was used as an ellagitannin adsorbing material. Glass column (Borosil, 2”x40”) packed with XAD-16 resin (resin bed height 10”) and itwas pre-washed with methanol. Packed column equilibrated with distilled water for 12 hr and vacuum aspirated. Obtained aqueous extract from fresh and fermented pomegranate peel passed through prepared column. The optimal loading volume of aqueous peel extract was 100±10 ml per column. Each loaded column were eluted with copious amount (1 Liter) of distilled water until the sugary pale yellow elute was clear in colour. Remaining water was removed from the resin by vacuum aspiration. Adsorbed ellagitannin in resin eluted with 100 ml solvent (Ethanol: Acetone:: 80:20) to yield a dark brown colour elute. To complete one cycle of elution per column was 5 min. Then residual solvent was removed under vacuum (-18 mbar pressure) and 40 $^{\circ}$C in vacuum drier (Shel Lab, USA) to yield a dark brown colouredellagitannin powder. Total ellagitannin powder was weighed and yield was calculated. The XAD-16 resin packed columns were regenerated by washing with distilled water and reused for purification of fresh and fermented peel extract.

2.7. Analysis of total ellagitannin content

Total ellagitannin content (TEC) in the extract was determined by using Folin-Denis ciocalteu reagent as described in AOAC method 952. 03 with slight modification. About 250 µl of extract was diluted with distilled water to 10 ml. Aliquots of 1 ml of diluted extract were mixed with 5 ml of 10 fold diluted Folin-Denis ciocalteu reagent. After 3 min, 4 ml of 7. 5% sodium carbonate was added. The mixture was allowed to stand for 30 min at 30 $^{\circ}$C before the absorbance was measured at 734 nm using UV-Vis spectrophotometer (Shimadzu UV-Vis-2600, Singapore). The total polyphenol content in the extract was calculated and expressed as gallic acid equivalent (GAE; g/100 g on dried basis) using a gallic acid (GA) (0-120 mg/l) standard curve. All samples were analyzed in triplicate.

2.8. Measurement of antioxidant activity

The antioxidant activity (AOA) of fresh and fermented peel extracts was measured in term of hydrogen donating or radical

scavenging ability using stable DPPH method according to the method proposed by Brand-Williams *et al*[2]. Aliquot of 250 µl of the extract was diluted with distilled water to 10 ml. Aliquot of 200 µl of samples were mixed with 2 ml of 100 µM DPPH methanolic solution. The mixture was placed in dark at room temperature for 60 min. the absorbance of resulting solution was then read at 520 nm using UV-Vis spectrophotometer (Shimadzu UV-Vis-2600, Singapore). The antiradical activity was expressed in terms of percentage reduction of the DPPH. The ability to scavenge the DPPH radical was calculated using the following equation:

AOA as DPPH (%) = $((A_0-A_1)/A_0)$ x100

Where A_0 is the absorbance of the control, and
A_1 is the absorbance of the sample

3. RESULT AND DISCUSSION

In Indian market 1 kg fresh pomegranate fruit cost is about Rs. 100 (NHB, India). One kg fresh pomegranate fruit of Bhagwa variety is containing 350 to 390 g of peel (including pith and carpellary membrane part). If we think about its cost is worth of Rs. 35-39 which is going to waste if not utilized. It is well known that this peel part is having hydrolysable ellagitannin exhibiting high antioxidant activity. This ellagitannincan be isolated and purified to get the pure ellagitannin powder.

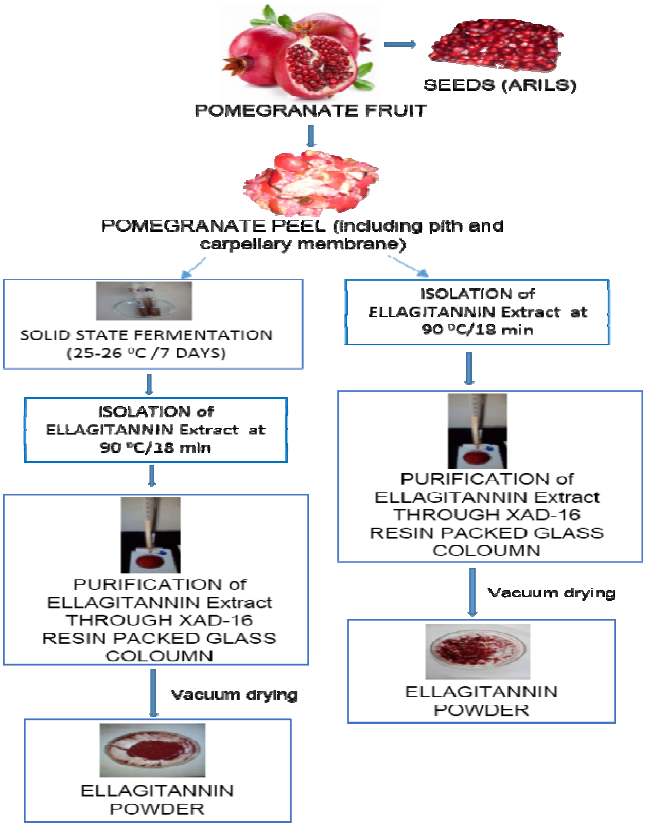

Fig. 1: Schematic diagram of ellagitannin production

3.1. Isolation of ETs extract from peel

Isolation of ETs has been carried out by two method (a) direct isolation from peel and (b) fermentation of fresh peel as shown in fig. 1. In first method fresh grounded peel was utilized and ellagitannin was extracted by using water as solvent. In second method fresh peel fermented with *Aspergillusniger* strain for 7 days and then isolated the ETs. During fermentation *A. niger* grows on the surface of peel and releases the tannase enzyme which break down the complex polyphenolic compound in to lower molecular weight polyphenolic compound which can be easily bound by adsorbent resins. Isolated ETs extract had shown good total ETs content (TEC) and respective antioxidant activity (AOA) as DPPH i. e. 42. 74 ± 1. 26 % and 63. 63 ± 2. 15 % as DPPH respectively in ETs extract from fresh peel. While ETs extract from fermented peel having TEC and AOA of 43. 20 ± 1. 37 % and 63. 89 ± 1. 88 % as DPPH respectively (Table. 1, Fig. 2). From above response it can be observed that both type of isolation method may give a desirable quantity and quality of ETs extract except time consuming factor in fermentation technique.

Table 1: Total ellagitannin content and antioxidant activity of peel extract and purified TEs powder

Sample	Parameters	
	TEC as GAE (% w/w, DM)	Antioxidant activity as DPPH (%)
Fresh Peel's ellagitannin extract	42. 74 ± 1. 26	63. 63 ± 2. 15
Fresh Peel's purified ellagitannin powder	91. 78 ± 1. 97	96. 21 ± 1. 72
Fermented Peel's ellagitannin extract	43. 20 ± 1. 37	63. 89 ± 1. 88
Fermented Peel's purified ellagitannin powder	89. 78 ± 1. 57	91. 16 ± 1. 60

3. 2. Purification of ETs Extract

ETs extract from peel has been purified through Amberlite XAD-16 resin packed column. XAD-16 resin is white translucent beads, nonionic macro reticular resin and polymeric adsorbent that adsorbs and releases ionic species through hydrophobic and polar interactions; usually used under isocratic condition. This resin can adsorbs hydrophobic compounds up to 40000 MW. It can be reused up to 100 times by regeneration with methanol, acetone, and isopropanol addition with water after each uses. ETs adsorbed in resin has been eluted with methanol: acetone (85:15) and obtained the dark brown colour elute and vacuum dried to convert it in to powder form. This ETs powder is dark brown coloured, bright and free flowing. It can be observed from Table 1 that the TEC and AOA in ETs powder from fresh peel is 91. 78 ± 1. 97 % and 96. 21 ± 1. 72 % as DPPH respectively while in ETs powder prepared from fermented peel has shown TEC and AOA 89. 78 ± 1. 57 % and 91. 16 ± 1. 60 % as DPPH

respectively. The AOA of ETs powder from fermented peel was slightly lower than ETs powder from fresh peel. It may be due to the oxidation degradation of phenolic compounds during fermentation. After purification of ETs extract from fresh and fermented peel, content of TEC and respective AOA increased in ETs powder. So, ETs powder may be considered as an ultimate product for its further application e. g. as bio preservatives, nutraceutical, additives and antioxidative agent in different food, pharma and cosmetics products.

3. 3. Economical aspect of ETs preparation process

Economical aspect of ETs preparation process has been estimated (Table 2) and found that it is very cost effective. Fresh pomegranate peel can be easily collected from juice industry free of cost and when we talk about its worth, i. e. of Rs. 35-39, because average price of 1 kg fresh fruit is Rs. 100 (NHB, India) and whole fruit is containing 35-39 % peel part. Grinding, blending and centrifugation process cost may be Rs. 50. Isolation and purification and solvent cost is Rs. 500 and this solvent can be recycled up to 70-80 % of its initial volume by distillation process. XAD-16 resin cost is Rs. 2400 per 100 g and it can be reused up to 100 times after regeneration. Glass column cost is Rs. 2500 which may be used for a long period unless it may break. Vacuum drying process may cost Rs. 60 after considering the electricity consumption units (Rs. 15/unit and operational cost per hr) at optimum temperature and pressure and considering the drying time (4 h). Overall ETs extraction process may cost approx. Rs. 5510 to process 1 kg fresh peel.

Table 2: Cost estimation and economical aspect of ellagitannin extraction process

S. No.	Material/Process	Quantity/No.	Estimated Cost (Rs.)	Economical Aspect
1	Fresh Pomegranate peel	1 kg	0	juice industry waste of worth Rs. 35-39 (1 kg fresh fruit avg. price is Rs. 100 and whole fruit is containing 35-39 % peel part, hence peel worths of Rs. 35-39/kg) available free of cost
2	Grinding, Extraction and other process	1 kg	50	may include the grinding,blending and centrifugation process
3	Extraction and purification solvent	500 ml	500	Can be recycled up to 70-80 % of its initial volume by distillation process
4	XAD-16 Resin	100 g	2400	can be reused 100 times for purification after regeneration with water
5	Glass column	5	2500	It can be used for long period until unless it not broken
6	Drying process for peel extract	1 liter	60	A vacuum trey dryer of 100 liter volume capacity can dried 5 lit solvent per 4 hr at optimum temp and pressure condition @ Rs. 15/hr operating cost (hence process cost for 4 hr would be Rs. 60)
Total cost (Rs.)			5510	

Table 3

S. No.	Yield and worth of ellagitannin Powder	
1	Quantity of purified Ets powder obtained from 1 kg fresh peel (g)	38. 2
2	Quantity of purified Ets powder obtained from 1 kg fermented peel (g)	37. 8
3	Cost of 1 g ellagitannin in international market (Rs.)	666
4	worth of purified ellagitannin (38. 2 g) powder (Rs.)	25441

For S. No. 3 source:http://www . scalarhealth. com/ el1 20 ca. html

3. 4. Yield and worth of ETs powder

Yield and worth of purified ETs powder has been estimated (Table 3) and found that purified ETs powder (37. 8-38. 2 g) can be obtained from 1 kg fresh pomegranate peel. Worth of 1 g of ETs powder is Rs. 666 in international market. Hence cost of purified ETs powder (38. 2 g) would be Rs. 25441, hence above process showing a good profitability for commercialization point of view.

4. CONCLUSION

A waste of juice industry can be processed for isolation of ellagitannin by above discussed method and can be prepared a highly valuable purified ellagitannin powder having appreciable amount of TEC (89. 78-91. 78 % as GAE) and AOA (91. 16-96. 21 % as DPPH). It can be used as bio preservatives, nutraceutical additives and ant oxidative agent in different food, pharma and cosmetics products. From 1 kg fresh peel approximately 37-38 g of purified ellagitannin powder can be produced of worth Rs. 25441 in International market.

REFERENCES

[1] Seeram, Navindra P. , et al. "In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. " *The Journal of nutritional biochemistry* 16. 6 (2005): 360-367.

[2] Negi, P. S. , et al. "Antioxidant and antibacterial activities of various seabuckthorn (Hippophaerhamnoides L.) seed extracts. " *Food Chemistry* 92. 1 (2005): 119-124.

[3] Vidal-Madjar, A. , et al. "Detection of oxygen and carbon in the hydrodynamically escaping atmosphere of the extrasolar planet HD 209458b. "*The Astrophysical Journal Letters* 604. 1 (2004): L69.

[4] Chidambara, M. K.; Reddy, V. K.; Veigas, J. M.; Murthy, U. D. Study on wound healing activity of Punicagranatum peel. *J. Med. Food* 2004**, 7:**256–259.

[5] Gracious, R. R.; Selvasubramanian, S.; Jayasundar, S. Immuno-modulatory activity of Punicagranatum in rabbits s A preliminary study. *J. Ethnopharmacol*. 2001, **78:** 85–87.

[6] Navarro, V.; Villarreal, M. L.; Rojas, G.; Lozoya, X. Antimicrobial evaluation of some plants used in Mexican traditional medicinefor the treatment of infectious diseases. *J. Ethnopharmacol.* 1996, **53**: 143–147.

[7] Tzulker, R.; Glazer, I.; Bar-Ilan, I.; Holland, D.; Aviram, M.; Amir,R. Antioxidant activity, polyphenol content, and related com-pounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. *J. Agric. Food Chem*. 2007, **55:** 9559–9570.

[8] Whitley, A. C.; Stoner, G. D.; Darby, M. V.; Walle, T. Intestinal epithelial cell accumulation of the cancer preventive polyphenol ellagic acids Extensive binding to protein and DNA. *Biochem. Pharmacol*. 2003, **66:** 907–915.

[9] Dai, J. and Mumper, R. J. Plant Phenolics: Extraction, Analysis and their antioxidant and Anticancer Properties. *Molecules* 2010, **15**: 7313-7352.

[10] Henning, Susanne M. , Piwen Wang, and David Heber. "Chemopreventive effects of tea in prostate cancer: green tea versus black tea. " *Molecular nutrition & food research* 55. 6 (2011): 905-920.

[11] Spigno, Giorgia, LorenzaTramelli, and Dante Marco De Faveri. "Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. " *Journal of Food Engineering* 81. 1 (2007): 200-208.

[12] Yilmaz, Y. , & Toledo, R. T. (2006). Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. J. of food composition and analysis, 19, 41-44.

[13] Pinelo, M. D. Fabbro, P. Marzocco, L. Nunez, M. J. &Vicoli, M. C. (2005a), optimization of continuous phenol extraction from *Vitisvinifera* byproducts. J. Food Chemistry, 92, 109-117

[14] Çam, Mustafa, NecattinCihatİçyer, and FatmaErdoğan. "Pomegranate peelphenolics: microencapsulation, storage stability and potential ingredient for functional food development. " *LWT-Food Science and Technology* 55. 1 (2014): 117-123.

[15] Patel, Chintan, et al. "Safety assessment of pomegranate fruit extract: acute and subchronic toxicity studies. " *Food and chemical toxicology* 46. 8 (2008): 2728-2735.

[16] Li, Jianke, et al. "Chemical fingerprint and quantitative analysis for quality control of polyphenols extracted from pomegranate peel by HPLC. " *Food Chemistry* 176 (2015): 7-11.

[17] National Horticulture Board, India, 2015

[18] Brand-Williams, W., M. E. Cuvelier, and C. L. W. T. Berset. "Use of a free radical method to evaluate antioxidant activity. " *LWT-Food Science and Technology* 28. 1 (1995): 25-30.

[19] A. O. A. C. , (2005). *Official Methods of Analysis,* 19th Edition, Association of Official Analytical Chemists. Washington D. C