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ORIGINAL ARTICLE

Chemo-profiling of anthocyanins and fatty acids present in pomegranate aril and seed grown in Indian condition and its bioaccessibility study

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Abstract The goal of the present study was to investigate the bioactive molecules (anthocyanins and fatty acids) present in the aril of pomegranate. Major anthocyanins present in the aril of pomegranate were identified by HRMS as delphinidin 3,5-diglucoside, cyanidin 3,5-diglucoside, pelargonidin 3,5-diglucoside, cyanidin 3-glucoside and delphinidin 3-glucoside. In-vitro study revealed that bioaccessibility of anthocyanin in duodenal condition was varied between 7.3 and 9.7%. Encapsulation enhances the bioaccessibility of both the phenolics to some extent in gastric as well as duodenal condition. Seed oil contains significant amount of unsaturated fatty acids especially ω-5 fatty acids. Geometrical isomers of ω-5 fatty acids were also identified by GC–MS. The spray dried anthocyanin formulation has potential for food application.

Keywords Pomegranate aril · Seed · Anthocyanins · HRMS characterization · Spray drying · Bioaccessibility

Introduction

Polyphenols are well known to be powerful antioxidants and they are abundantly present in fruits and vegetables (Hertog et al. 1997). Investigations showed that these polyphenols are responsible for the prevention of various diseases including digestive tract infection (Serafini et al. 2002). Investigations reported that most of the polyphenols present in human diet are not as active as a nutraceutical because of its limitations like limited absorption in human digestive system (Saura-Calixto et al. 2007; Heim et al. 2002).

Pomegranate (*Punica granatum*) aril is a source of powerful antioxidant, anthocyanin. Glycosides of cyanidin, pelargonidin, peonidin, delphinidin, petunidin, and malvidin are the major anthocyanins present in plants. Based on earlier research reports, pomegranate juice is known to be a major source of 3-glucosides and 3,5-diglucosides of delphinidin, cyanidin, and pelargonidin, apart from other polyphenolics (Du et al. 1975). Relative constituent of individual anthocyanins and its glucosylated form vary with the climatic condition.

With the advancement of analytical techniques, especially hyphenated chromatographic and spectrometric techniques with high accuracy, elucidation of chemical structure and determination of its role in different pathways have become easier for understanding. Liquid chromatographic separation followed by high accuracy detection by high resolution mass spectrometry (LC-HRMS) is key in identification of plant secondary metabolites (De Vos et al. 2007).

Apart from the anthocyanins, seed contains significant amount of oil. Edible oil with conjugated fatty acids are known to be active physiologically. Conjugated linolenic acids (CLNA) are one of the important fatty acid that have

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significant nutraceutical properties (Nagao and Yanagita 2005). Different geometric and positional isomers of CLNA were reported in different seed oils. Pomegranate seed oil is reported to have ω-5 fatty acids, apart from other mono and di-saturated fatty acids (Kaufman and Wiesman 2007).

In our opinion, there is no report of anthocyanin characterization of the pomegranate cultivars grown in India. Limited reports are available on the bioaccessibility of these anthocyanins in human system. Further, encapsulation of these polyphenols in a food matrix might change the release behavior of these phytochemicals and thus may interfere with the bioaccessibity. So, the major aim of the present study is to provide new insights into bioaccessiblity of the anthocyanin in its crude extract, purified and formulated form. HRMS characterization of anthocyanins present in the pomegranate fruit will be helpful in metabolite profiling upon climatic variations of Indian subcontinents. The study also aimed to characterize the fatty acids present in the seed oil of the cultivar, especially conjugated linolenic acids. The study deals with the extraction, purification and characterization of anthocyanins and fatty acids from pomegranate aril and seed. Bioaccessibility of anthocyanin was also evaluated in purified and encapsulated form.

Experimental section

Sampling of pomegranate cultivars

For extraction of anthocyanin and seed oil, pomegranate fruits (*Bhagwa* variety) was selected and procured from a local market in Delhi. Peels, arils and seeds were separated and stored at − 40 °C.

Chemicals

HPLC grade solvents (methanol, acetonitrile) were procured from Merck® India Pvt. Ltd. Resins, XAD-7 (Amberlite®) and standard cyanidin-3-glucoside (C_3G) were procured from Sigma-Aldrich, USA. Pepsin enzyme, pancreatin, bile salts, cellulose dialysis tubing required for the study were purchased from Sigma Aldrich Ltd., Louis, USA.

Extraction

Extraction of anthocyanin

Extraction of anthocyanins was executed by following the methodology already standardized in the laboratory (Pradhan and Saha 2016). Arils of pomegranates were separated and squeezed to get the juice. The juice was acidified with 0.1% HCl. The juice was evaporated to dryness using rotary evaporator (< 45 °C) to get the anthocyanin rich extract as dark coloured viscous mass. After removal of juice, seeds were separately washed and dried for the extraction and characterization of its fixed oil.

Purification of anthocyanin The dried anthocyanin rich extract was subjected to purification by passing through a macroporous adsorption resin (XAD-7) column. Initially the concentrate was loaded in the column, the column was eluted with water twice so as to evacuate sugars and different other phenolics. After that the column was eluted with ethanol to get the fractions of column bound anthocyanins. Elution of ethanol proceeded till the column eluent turned out to be practically colourless. Ethanol was evaporated utilizing rotary evaporator under vacuum (< 40 °C) and the residue was dissolved in water for prefreezing. The sample was lyophilized on a lyophilizer to get fine anthocyanin crystals.

Analysis of anthocyanin by HPLC HPLC system was used for the analysis of purified anthocyanin using C_{18} column (Hypersil; 250 mm × 4.6 mm × 5 µ; Thermo Fischer Scientific) for the better separation. Multistep linear gradient solvent system comprising of water (0.1% TFA; solvent A) and water: ACN: TFA (53:46:1 v/v; solvent B) was used for the anaysis. The gradient elution profile was as follows: 0 min 80% A, 26 min 60% A, thereafter to 20% in 4 min and stay constant for 10 min and then back to initial composition. The injected volume was 20 µL and the flow rate was 0.6 mL min^{-1}. The chromatogram was recorded at wavelengths of 520 nm. Empower 2® was used as operating system for data analysis. Retention times and UV–visible spectral fingerprint of the standard and compounds in extract were used for preliminary identification of individual anthocyanins. Total anthocyanin was also calculated as cyanidin-3-glucoside equivalent after considering all the peaks together.

HRMS characterization of anthocyanin The purified anthocyanin was analyzed by UHPLC high resolution mass spectroscopy (HRMS/ESI-Orbitrap) for the characterization. Conditions were same as mentioned in the earlier (Sarkar et al. 2018).

Extraction of seed oil

Pomegranate seed oil was extracted by following AOAC (AOAC method 945.16; Association of Official Analytical Chemists) method using petroleum ether (40–60 °C) as the solvent in a Soxhlet apparatus.

Characterization of oil For characterization of fatty acids present in pomegranate seed oil, fatty acid methyl esters were prepared. It was prepared upon addition of methanol in few drops of oil and sulfuric acid, followed by incubation in a water bath at 65 °C for 1 h. The methyl esters were extracted after partitioning with hexane and it was analyzed using gas chromatograph–mass spectrometry (GCMS). GC–MS analysis was carried out using a GC (7890A; Agilent Technologies) connected to a triple axis HED-EM 5975C mass spectrometer (Agilent Co., USA). Analysis was done by an HP-5MS column (30 m × 0.25 mm; 0.25 µm, Agilent Co., USA). 1 µL sample was injected using split mode of injection. The carrier gas (Helium) flow was set at 1 mL min^{-1}. The used GC–MS conditions for the analysis is described as follows: the oven temperature was initially held at 40 °C, then raised with a gradient of 5 °C min^{-1} and held for 1 min. Next temperature enhancement was done with a gradient of 5 °C min^{-1} and held for 5 min. The total run time was 54 min. Other instrument settings were as follows: 250 °C interface temperature, 200 °C ion source temperature, and electron impact ionization (EI) at 70 eV. The MS acquisition parameters were: ion source 180 °C, electron ionization 70 eV, full scan mode (50–550 mass units), transfer line temperature 280 °C, solvent delay 3 min, and E.M. voltage 889. The ionization energy was 70 eV with a scan time of 1 s and mass range of 20–500 amu. Compounds were identified by matching their mass spectra. NIST (National Institute of Standards and Technologies) Mass Spectra Library was used as a reference for identifying the essential components.

Microencapsulataion of anthocyanin

For preparation of microcapsule, maltodextrin was used as carrier for the encapsulation. For encapsulation, maltodextrin (20 g) and anthocyanin (2 g) were mixed in 125 mL of water and then the sample was fed into a spray drier (Labultima, Mumbai), where the mixture was nebulized with a pressure ranging from 1.97 to 2.38 kg cm^{-2} under vacuum (184 mm). The inlet temperature was maintained about 151.4–170.0 °C and the outlet temperature was at about 61.7 °C. The resultant powdered formulation was collected from different cyclone chambers and stored in a sealed container (− 20 °C) for further analysis.

Estimation of encapsulation efficiency

For the determination of encapsulation efficiency, surface anthocyanins were estimated after extraction of 200 mg of encapsulated materials with 2 mL of ethanol for 1 min. Amounts of extracted anthocyanins were quantified as described in earlier section. Then encapsulation efficiency was calculated for both the compounds separately.

Scanning electron microscopy

Outer surface morphology of the encapsulated formulations (blank and anthocyanin loaded) were taken with Zeiss EVOMA10 scanning electron microscope. Sample preparation was done by mounting approximately 0.5 mg of material in powdered form on an aluminium stub followed by sputter-coating with palladium layer.

Bioaccessibility study

The bioaccessibility studies were carried out to get an idea about the gastrointestinal absorption of the compounds, which also provide a significant correlation between in vitro and in vivo measurements (McDougall et al. 2005). It included two steps:

(1) Pepsin/HCl digestion:
The sample (700 mg) was digested with 20 mL of acidified water (pH 1.7, adjusted with 11.8 (N) HCl) and 25.2 mg of pepsin followed by incubation at room temperature (35 ± 2 °C) with shaking at 100 rpm in magnetic stirrer for 2 h.

(2) Neutralization of the digested sample mixture in the mimic of gastrointestinal tract:
After 2 h of digestion, in the digested sample mixture 18 mg pancreatin (4.5 mL of 4 mg mL^{-1} pancreatin, Sigma Aldrich Ltd., Louis, USA) and 500 mg bile salts were added. In a dialysis tube (MWCO 12400, 40 mm flat width with 99.99% retention, used as a mimic of gastrointestinal tract) 5.6 mL of 0.75 (M) $NaHCO_3$ (titratable acidity) was added and placed in that digested sample mixture for 2 h. Then 0.75 mL of aliquot from the inside mixture of the dialysis tube (expressed as IN) was taken out, checked for the pH (pH-7.25), and then analyzed in HPLC after 50% dilution with water.

Results and discussion

Characterization

Characterization of anthocyanin

After HPLC analysis, considering all the constitutive peaks of anthocyanin, total anthocyanin content was recorded as 37 mg 100 mL^{-1} of aril juice. Our result is consistent with the earlier results (Fawole and Opara 2013) and is within

the range of those previously published (Melgarejo et al. 2000).

Recent literature reported characterization of polyphenols by HRMS data using both positive and negative mode (Sun et al. 2013; Riffault et al. 2014; Michel et al. 2015; Salerno et al. 2016).

Orbitrap is known for its high mass accuracy ($\Delta m <$ 2–3 ppm) and elemental composition. For reducing the number of hits, mass tolerance was set at below 5 ppm. Major resolved peaks in TIC were selected and their elemental composition was recorded. Molecular ion peak followed by MS/MS spectra with selected precursor ions helped to identify the structures of the compounds.

Anthocyanin rich extract was obtained after concentration and purification by adsorbent resin. The extract upon HPLC analysis yielded five major peaks, which showed absorption maxima of 504.2–522.5 nm (Fig. S.1). HPLC profile revealed two major and three minor peaks in the chromatogram. Although other smaller intensity peaks were also visible in the chromatogram.

The first major peak, eluted at retention time of 19.824 min, showed UV maxima of 521.3 nm. HRMS analysis revealed that the experimental mass of the peak 1 was 627.1549, which was closer to the theoretical mass of Delphinidin-3,5-diglucoside with a error mass value of − 1.17. So, the compound was characterised as Delphinidin-3,5-diglucoside (Table 1).

Second major peak (R_t 22.053 min) in the LC chromatogram was maximum in concentration. The peak had a molecular ion peak at m/z 611.1631 with a base peak of m/z 287. With the error values of 3.07, the peak was identified as cyanidin-3,5-diglucoside with empirical formula of $C_{27}H_{31}O_{16}$.

Peak 3 and 4 eluted at a close interval with R_t value of 24.416 and 24.532 min. UV–Vis spectrum of these two peaks revealed the λ_{max} value of 504.2 and 518.8 nm, respectively. Molecular ion peak of peak 3 and 4 were recorded at m/z 595.1645 and 465.1033. Base peak of these two peaks were m/z 271 and 303 confirmed the structures as Pelarginidin-3,5-diglucoside and Delphinidin-3-glucoside, respectively. Error mass value was calculated as − 2.02 and − 1.93 for those peaks (Table 1).

Last peak was eluted at retention time of 27.009 min. The peak was characterised as cyanidin-3-glucoside after scrutinizing its molecular ion peak value (m/z 449.1092) and error mass value of 1.78. Confirmatory base peak of cyanidin and its λ_{max} value of 517.6.

Major anthocyanins present in pomegranate from Iran were delphinidin 3-glucoside, delphinidin 3,5-diglucoside, pelargonidin 3-glucoside, pelargonidin 3,5-diglucoside, cyanidin 3-glucoside and cyanidin 3,5-diglucoside (Alighourchi et al. 2008). In another study, nine anthocyanins were reported to be present in the aril, amongst which cyanidin-3,5-diglucoside was the major compound (Fischer et al. 2011). MALDI-TOF MS was used by Afaq et al. (2005) for the characterization of six anthocyanins present in the aril of pomegranate. Reported anthocyanins were pelargonidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-glucoside, pelargonidin 3,5-diglucoside, cyanidin 3,5-diglucoside and delphinidin 3,5-diglucoside.

Characterization of oil

Transesterification was done to covert fatty acids in oils to fatty acid methyl esters (FAME). Fatty acid compositions present in pomegranate seed oil are presented in Table 2. GC–MS analysis demonstrated wide distribution of fatty acids. It started from palmitic acid (16:0) to long chain fatty acid (21:1). Saturated fatty acid constituted up to 9.3%, whereas, unsaturated fatty acids constituted 90.7%. Out of unsaturated fatty acids, 11.1% were mono-unsaturated, 11.6% were di-unsaturated and 68.1% were tri-unsaturated (Fig. 1). Fragmentation pattern of FAME after GC–MS analysis revealed characteristic peak of saturated, mon-unsaturated, di-saturated and tri-unsaturated fatty acids were m/z 74, 55, 67 and 79. Contribution of these characteristic peak in these different fatty acids were presented in Fig. 2. Contribution of m/z 67 and 69 were very

Table 1 Retention time, molecular ion (in positive mode) and error mass of HRMS analysis of identified compound present in resin purified extract

Peak number	R_t (min; HPLC-PDA)	Theoretical mass	Experimental mass	Error mass (Δ, ppm)	Chemical formula	Tentative identification
1	19.824	627.1556	627.1549	− 1.17	$C_{27}H_{31}O_{17}$	Delphinidin-3,5-diglucoside
2	22.053	611.1612	611.1631	3.07	$C_{27}H_{31}O_{16}$	Cyanidin-3,5-diglucoside
3	24.416	595.1657	595.1645	− 2.02	$C_{27}H_{31}O_{15}$	Pelarginidin-3,5-diglucoside
4	24.532	465.1033	465.1024	− 1.93	$C_{21}H_{21}O_{12}$	Delphinidin-3-glucoside
5	27.009	449.1084	449.1092	1.78	$C_{21}H_{21}O_{11}$	Cyanidin-3-glucoside

Table 2 Fatty acid composition of pomegranate seed

No.	R_t (min)	Compound	Relative composition (%)
1	32.881	C16:0	5.30
2	36.085	C18:2 (9,12-octadecadienoic acid)	11.56
3	36.188	C18:1 (9-Octadecenoic acid)	8.34
4	36.280	C18:1 (7-Octadecenoic acid)	1.23
5	36.646	C18:0	3.31
6	38.614	C18:3	22.72
7	38.666	C18:3	12.81
8	38.912	C18:3	18.27
9	39.227	C18:3	2.29
10	39.370	C18:3	12.00
11	39.684	C21:1	1.52
12	40.096	C21:0	0.64
13	Total saturated		9.25
	Total unsaturated		90.74

less in saturated fatty acids, whereas, m/z 74 in di- and tri-unsaturated fatty acids were very less as compared to other three peaks.

Interestingly, five peaks of linolenic acid showed molecular ion peak at m/z 192.1. Total linoleic acid content was calculated by adding all the five peaks corresponds to m/z of 192. Our results are in agreement with earlier reports (Kaufman and Wiesman 2007; Fadavi et al. 2006; Suzuki et al. 2001). There were five different peaks of linolenic acid (18:3) as extracted from the Wiley-NIST library. Earlier studies reported only four isomers of the fatty acids (Kaufman and Wiesman 2007). In earlier study, first eluted isomer of linolenic acid was of lion share. In the present case, distribution of the isomers were comparatively even distributed. All the five peaks have similar mass fragmentation pattern, with differences in their intensities of the fragmentation ion peaks. Fragmentation pattern of all the five peaks were depicted in Fig. S.2. All the peaks have molecular ion peak at m/z of 292 with their fragments at m/z at 79, 91 and 93. Intensities of fragment ion peaks with same molecular ion peak confirmed the isomers of linolenic acid. Earlier investigations were done in reference to identification of linolenic acid isomers (Hornung et al. 2002; Kaufman and Wiesman 2007; Özgül-Yücel 2005; Suzuki et al. 2001). In the present study, geometric isomers of linolenic acid was identified based on mass spectrum of the GC resoluted peaks and earlier reports.

Constitutive fatty acids of pomegranate seed oils were reported to be punicic acid (9-*cis*, 11-*trans*, 13-*cis*), α-eleostearic acid (9-*cis*, 11-*trans*, 13-*trans*), catalpic acid (9-*trans*, 11-*trans*, 13-*cis*) and β-eleostearic acid (9-*trans*, 11-*trans*, 13-*trans*). Takagi and Itabashi (1981) and Özgül-Yücel (2005) both reported three isomers of CLNA, in which punicic and catalpic acid were common. Former reported presence of α-eleostearic acid whereas β-eleostearic acid was reported by the later. Suzuki et al. (2001) reported all four geometric isomers of CLNA. Almost all the studies, punicic acid was dominant in concentration. Kaufman and Wiesman (2007) reported presence of 74–92% punicic acid out of total CLNA. Takagi and Itabashi (1982) reported 96.5% of punicic acid out of total conjugated octadecatrienoic acid. Differential reporting in types of CLNA isomers present in pomegranate oil was recorded. It might be ambiguity or climatic variations in the composition. In the present study, punicic acid content was reported to be 33.4% of 18:3 fatty acids. Variations might be due to influence of climatic condition and also might be analytical advances led to better separation of the isomers. By comparing earlier data, other three isomers were tentatively identified as α-eleostearic acid, catalpic acid and β-eleostearic acid. Fifth isomer could not be identified with the present state of knowledge.

Microencapsulation

Average encapsulation efficiency of anthocyanins in maltodextrin encapsulated formulations was 82.9%. Similar result has been reported by Robert et al. (2010), where encapsulation efficiency of maltodextrin encapsulated ethanolic extract formulation was ranged between 52.9 and 82.8%. Our result was also in agreement with the report by Saenz et al. (2009). Our result was consistent with the report by Robert et al. (2010). The study reported 89–100% encapsulation efficiency of anthocyanin in maltodextrin, when pomegranate juice was encapsulated in maltodextrin. Idham et al. (2012) reported 99.7% encapsulation efficiency of Hibiscus anthocyanin in maltodextrin carrier. Mahdavi et al. (2016) reported encapsulation efficiency of barberry anthocyanins were in the range of 86–93%, when maltodextrin was used as carrier.

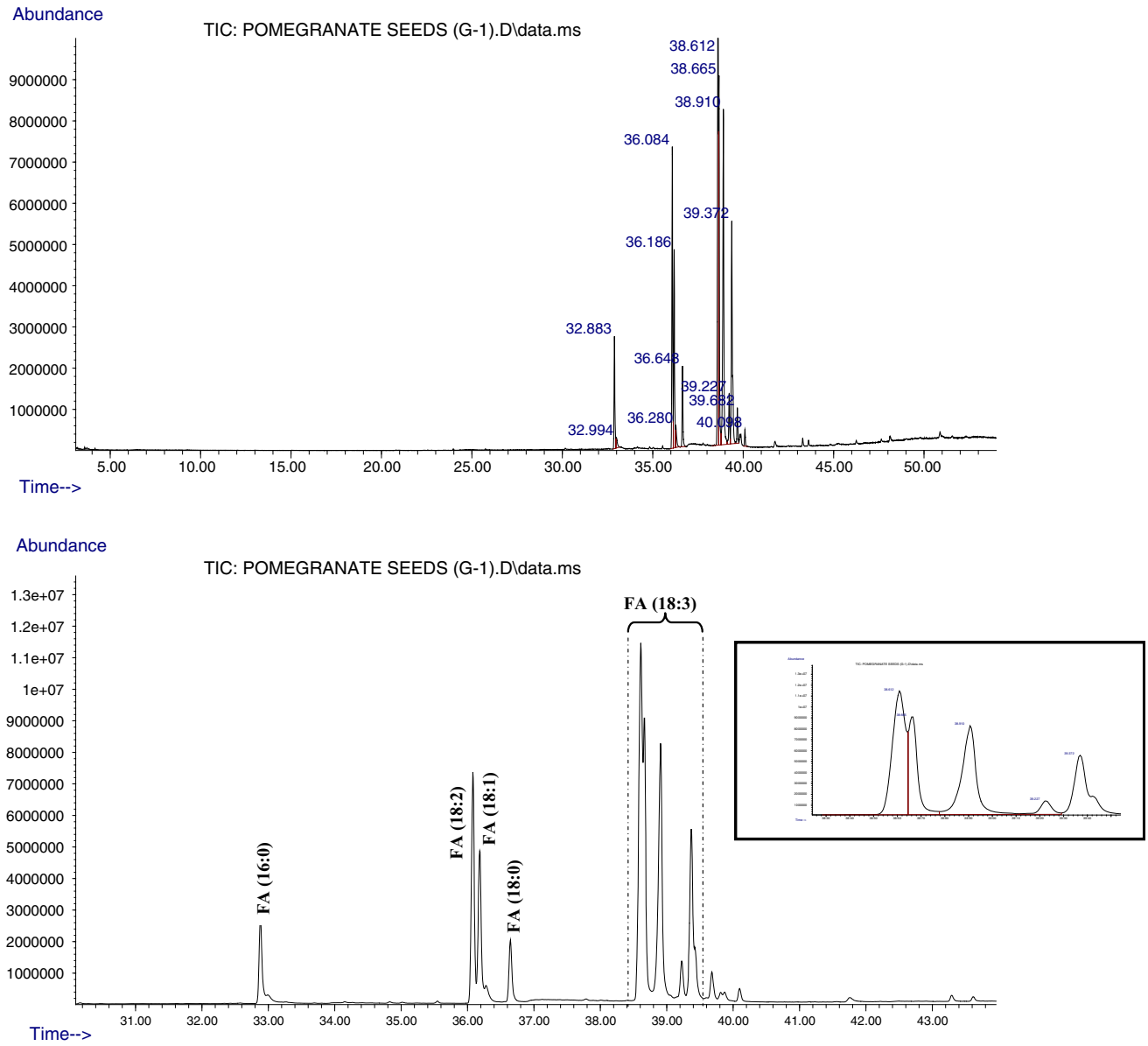

Fig. 1 GC–MS chromatogram of pomegranate seed oil

Figure 3 depicts the surface morphology of anthocyanin encapsulated formulation. Surface morphology of microcapsules was irregularly spherical in shape and shrinken in structure. Extensively dented surface might be attributed to the shrinkage of the particles during drying. Similar type of SEM picture was reported in maltodextrin microcapsules (Robert et al. 2010; Diaz Sanchez et al. 2006; Cai and Corke 2000; Tonon et al. 2008).

According to Nijdam and Langrish (2006), hollow particle cannot deflate when the drying temperature is sufficiently high as moisture is evaporated very quickly and the skin becomes dry and hard. On contrary, when the temperature is lower, the outer surface remains moist for longer time and it can deflate and shrivel as it cools.

Bioaccessibility

The result reported in Fig. 4 depicts that gastric and duodenal digestion of anthocyanins after evaluation by using in-vitro model simulating gastro intestinal condition. Amount of bioaccessible anthocyanins was ranged between 7.3 and 9.7%. In general, absorption in the human gut after digestion i.e. bioaccessibility of any compound depends on number of variables like molecular structure and its intrinsic properties as well as other components present along with the bioactive compounds (Lipinski et al. 2012). These factors dictates the uptake and concentration in blood as well as in cell. Mostly, concentration of the bioactive compound(s) in gastric and duodenal level

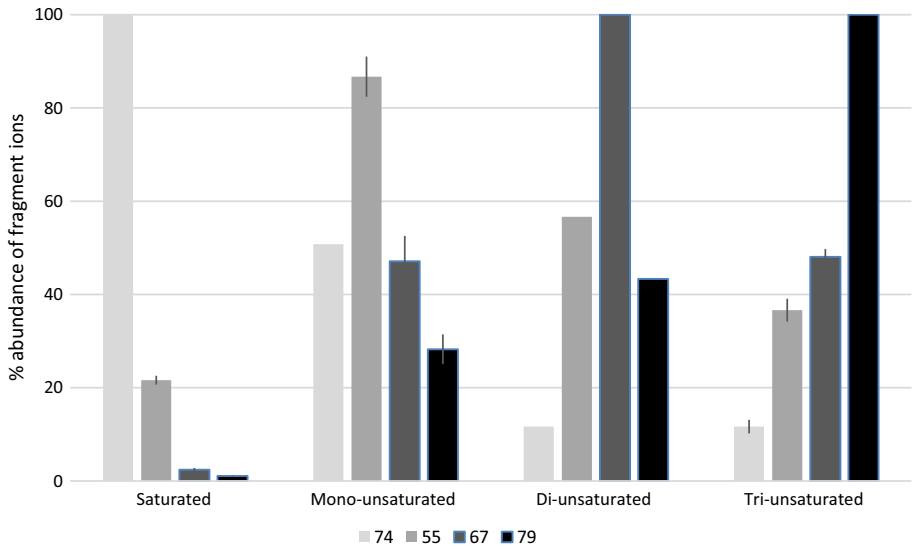

Fig. 2 Abundance of m/z 55, 67, 74 and 79 in saturated, mono-, di- and tri-unsaturated fatty acid mass fragmentation

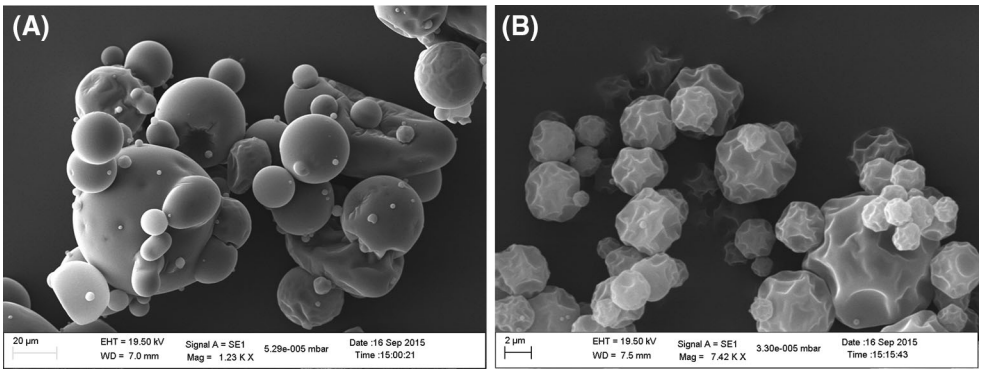

Fig. 3 Micrograph of maltodextrin (**a**) and spray dried anthocyanins (**b**)

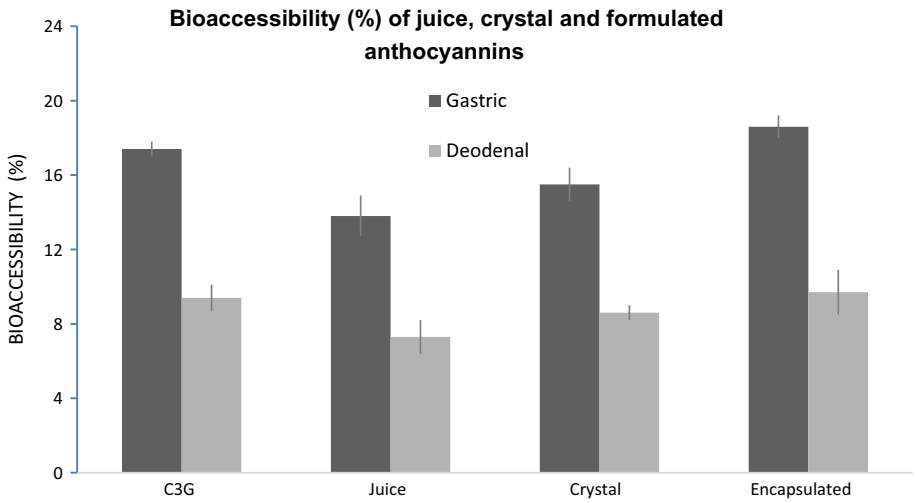

Fig. 4 Bioaccessibility (%) of juice, crystal and encapsulated anthocyanin extracted from pomegranate aril

determines the bioaccessibility. Number of studies reported the bioaccessibility of anthocyanin in different anthocyanin (Bobrich et al. 2014; Sengul et al. 2014) and there are few reports on the bioaccessibility of anthocyanin entrapped inside a structural barrier (Carrillo et al. 2017). Bioaccessibility of masticated black carrot anthocyanin was reported to be 23%, whereas, it was 10.6% when anthocyanin

was evaluated in jam and marmalade matrix (Kamiloglu et al. 2015).

Our result is in consistent with the report by Liang et al. (2012). The investigation reported 7.3% of mulberry phenolics as bioaccessible. Bouayed et al. (2011) reported dialysability of phenolics through cellulose membranes was about 25%. The study concluded that bioaccessible phenolics in the gastrointestinal tract were present both in soluble and bound form, such as proteins or polysaccharide conjugates (Manach et al. 2004).

Bioaccessibility of anthocyanin in the form of juice, purified crystal and formulated product was presented in Fig. 4. Bioaccessibilty was maximum in formulated samples followed by purified crystal and least in juice. Positive control, cyanidin-3-glucoside was comparable with these treatments. Bioaccessible anthocyanin in duodenal condition was varied between 7.3 and 9.7%. After gastric digestion, 18.6% anthocyanins were present in case of encapsulated material whereas, dialysability of anthocyanin in duodenal condition was 9.7%.

Any nutrient that bypassed the semi-permeable membranes connected to the jejunum and ileum compartments represented as bioaccessible. Nutrient/compound present in the ileal efflux normally delivered to the colon for absorption. Kamiloglu et al. (2015) reported dialysed anthocyanin fraction of black carrot was in the range of only 0.1–10.6% of the initial anthocyanin content. Ribnicky et al. (2014) reported that variable bioaccessibility of individual anthocyanins. The result showed that malvidin-containing anthocyanins had the highest relative bioaccessibility on average, followed by cyanidin, petunidin and delphinidin-containing anthocyanins.

Anthocyanins, in general were stable under the gastric conditions, but a significant decline after the pancreatic digestion was recorded. Around 10% of anthocyanins were detected in the IN samples (Fig. 4). It was well known that anthocyanins present in equilibrium of four molecular species. Under acidic condition (pH < 3) colored basic flavylium cation exists and three secondary structures were formed after the increase in pH values. At higher pH, anthocyanin equilibrates between the quinoidal bases, the carbinol pseudobase, and the chalcone pseudobase forms. Low detection of anthocyanins could be attributed to the transformation of the flavylium cation to the colorless chalcone pseudobase at the pH of intestinal environment (pH 7.5–8.0). With the enhancement of pH, ring fission occurs between ring B and C of anthocyanin with formation of ionized chalcones. Further, co-precipitation of anthocyanin upon acidification also responsible for the lower recovery of anthocyanin. Earlier similar observations were made by McDougall et al. (2005) and Liang et al. (2012).

Conclusion

The present study provides useful information about the bioaccessibility pattern of anthocyanins in the form of crude extract, purified extract and encapsulated formulation, after evaluation by in-vitro gastrointestinal model. The study demonstrated that encapsulation of these phenolics in maltodextrin carrier improved the bioaccessibility of both type of phenolics in in-vitro model system. Anthocyanins were characterised by HRMS with error mass value below 3 ppm confirms their structures. Fatty acid profiling was also done for the seed oil pomegranate and found the presence of ω-5 fatty acid. Further research efforts has to be done in this direction utilizing in-vivo model to reach to a comprehensive conclusion.

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