Pak. J. Biochem. Mol. Biol., 2023, 56(4):157-169

Research Paper

Metabolomic Characterization of Mango Cultivars Grown in Pakistan

Sumbal Saeed[1] and Huma Jawed[2*]

[1]Department of Pharmacy, Salim Habib University, Karachi, Pakistan.

[2]Department of Biosciences, Mohammad Ali Jinnah University, Karachi, Pakistan.

[*]Corresponding Author: Dr. Huma Jawed (drhuma@jinnah.edu).

ABSTRACT

Mango fruit is a rich source of various macro and micro-nutrients. It has been reported to possess numerous polyphenolic secondary metabolites. Major mango polyphenols identified in terms of respective free-radical scavenging ability and concentration in fruit are mangiferin, quercetin, kaempferol, catechins, rhamnetin, anthocyanins, methyl gallate, gallic acid, ellagic acids, benzoic acid, and protocatechuic acid. Pakistan is a commercial producer of 25 to 30 mango varieties. However, limited research done in the arena of variety-specific phytochemical content analysis of mango cultivars grown in Pakistan. The present study analyzes polyphenolic metabolites of 3 mango cultivars namely Dussehri, Sindhri, and Chaunsa using RP-HPLC. Metabolite analysis revealed that Sindhri mango fruit contained a diverse array of polyphenolics compared to the other 2 varieties tested. We obtained well-separated peaks in chromatograms at 260 nm and 280 nm. HPLC chromatograms of Dussehri fruit at 260 nm and 280 nm significantly differed from Sindhri and Chaunsa fruit chromatograms which were superimposable. The data obtained in this study provided novel information regarding mango cultivar-specific phenolic metabolites.

KEYWORDS: Mango Cultivars; Polyphenols; Langra; Chaunsa; Dussehri

INTRODUCTION

Mango is an important fruit crop in tropical and subtropical regions. More than 100 mango varieties grow in Pakistan. However, Sindhri and Chaunsa are among the major mango cultivars due to the rich taste and flavor of the fruits of these cultivars. Mango fruit breeding is not much known or established protocol in the country and generally open pollination is responsible for chance seedling. Tissue culturing and germplasm are relatively new concepts to cultivars. Mango hybrid technologies have been searched to boost the industry of mango which faces a decline due to high water table, unbalanced fertilization, soil salinity, and insufficient plant protection measures. The spread of fungal diseases and destructive pests such as *Drosicha mangiferae* remains to be among the major threat to mango plants [1].

HPLC is a useful analytical tool in phytochemistry for structural and functional analysis of plant metabolites in time time-efficient manner. The technique has found to be yielding satisfactory results in identification and separation polyphenols, amino acids, lipids, nucleic acids, carbohydrates, proteins and other bio-active molecules [2].

This study is dedicated to gaining novel insights into the polyphenolic content of Pakistan's mango varieties. Through this study, we aim to evaluate differences among Dussehri, Sindri, and Chaunsa mango varieties based on specific polyphenolic contents and to provide variety-specific chromatographic data at various wavelengths.

MATERIALS AND METHODS

Mango Pulp Polyphenol Extraction

Dussehri, Sindhri, and Chaunsa mango fruit varieties were purchased locally and pulp was separated from fruit peel and seed. Pulp of each variety was stored in labeled falcons at -4^{o}C.

10 grams pulp of each variety was weighed and was subjected to extraction through

literature reported method of maceration. The solvent combination used for polyphenol extraction from pulp of each variety was; ethanol (AnalaR, purity 99.7-100%): methanol (AnalaR, Purity 99.8%): acetonitrile (Daejung, purity 99%) (1:1:1) V:V:V total volume of 30ml. Each sample of separate mango variety was given 20 minutes for maceration with mild stirring at regular intervals. After the given time each sample was filtered through cheesecloth and then Whatman paper to remove the solid pulp pieces.

After filtration, each extract was subjected to drying using a rotary evaporator (STUART RE300P). Evaporation was carried out at 40 $^{\circ}$C under reduced pressure till complete solvent evaporation. Methanol (AnalaR, Purity 99.8%) (1:1) was used to dissolve the dried sample from the rotary round bottom flask. Each extract was then centrifuged to remove pectin at 2000 x g for 10 mins at 10 ^{o}C. After pectin removal samples were re-evaporated to remove organic solvents using the same rotary evaporator. HPLC-grade water was used in enough quantity to take out dried extract from the rotary round bottom flask. The final aqueous extract was then stored at -4^{o}C till further.

HPLC Analysis

For high-performance Liquid chromatography system, (Shimadzu model SPD 20A detector, and LC 20AT Pump A and Pump B) and C18 column (00B-4097-Z0, Prodigy porous silica solid support, particle size 5µm LC column 50 x 4.6mm) reverse phase column was used during this study. The injection volume for each sample was 20 µL. Binary gradient was used for polyphenol elution for which 2 solutions were prepared. Solution A, equilibration solution containing acidified water and acetic acid (Daegunj, 99.0-101.0% purity) 68:2 V/V. Water was acidified using 5ml of the highest purity hydrochloric acid (analytical grade) for preparation. Solution B, elution solution, contained distilled-deionized filtered water, acetonitrile (Daejung, 99% purity), and acetic acid (Daegunj, 99.0-101.0% purity) 68:30:2 V/V/V. HPLC software was programmed to gradually increase the concentration of solution B from 0 to 60% during time 5 to 30 minutes. From time 0.02 mins when controller starts to 5 mins B was kept 0%. At 5 mins solution B was programmed to increase gradually reaching 60% till 30 mins. To return to the initial concentrations of system concentration of solution B was commanded to decrease from 30 to 36 mins from 60 to 0% meanwhile solution A again returned to 100%. RP-HPLC was carried out 220 nm, 240 nm, 260 nm, 280 nm, 300 nm, 320 nm, 360 nm, 380 nm, and 400 nm.

RESULTS

We have characterized the fruit metabolome of three commercial mango cultivars using RP-HPLC. For this purpose, polyphenolic extracts of mango fruit pulp of mango cultivars were prepared followed by HPLC (Figure 1). The chromatograms presenting peaks of various polyphenolic compounds in extracts of Dussehri, Sindhri, and Chaunsa varieties at 260 nm are shown in figure 2. Chromatograms at this wavelength have shown well-separated peaks. The Chromatogram of dussehri fruit extract was significantly different from other varieties. HPLC peaks at 20 and 27 mins were common in chromatograms of all 3 extracts.

The chromatograms presenting peaks of various polyphenolic compounds in extracts of Dussehri, Sindhri and Chaunsa varieties at 280 nm are shown in figure 3. The Chromatogram of dussehri fruit extract at 280 nm was significantly different from the other 2 chromatograms which appeared to be superimposable. In the chromatograms of all extracts, there were no common HPLC peaks detected at 280 nm.

Likewise, the chromatogram presenting peaks of various polyphenolic compounds in extracts of Dussehri, Sindhri and Chaunsa varieties at 320 nm are shown in figure 4.

Overlayed HPLC chromatograms of phenolic extract of Dussehri mango at 220, 240, 260, 280, 300, 320, 340, 360, 380, 400 nms are shown in figure 5.

Overlayed HPLC chromatograms of phenolic extract of Sindhri mango at 220, 240, 260, 280, 300, 320, 340, 360, 380, 400 nms are shown in figure 6.

Overlayed HPLC chromatograms of phenolic extract of Chaunsa mango at 220, 240, 260, 280, 300, 320, 340, 360, 380, 400 nms are shown in figure 7.

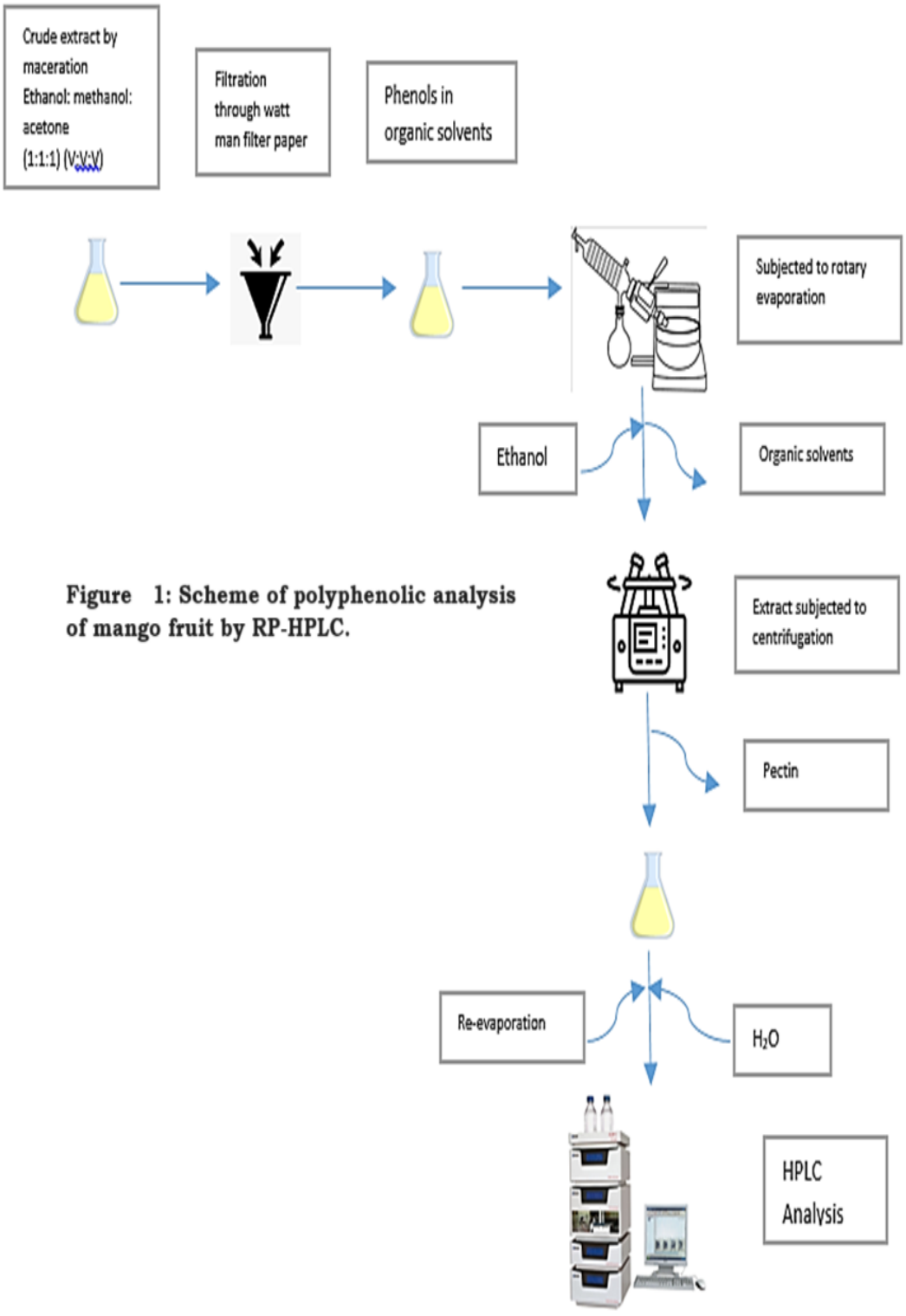

Figure 1: Scheme of polyphenolic analysis of mango fruit by RP-HPLC.

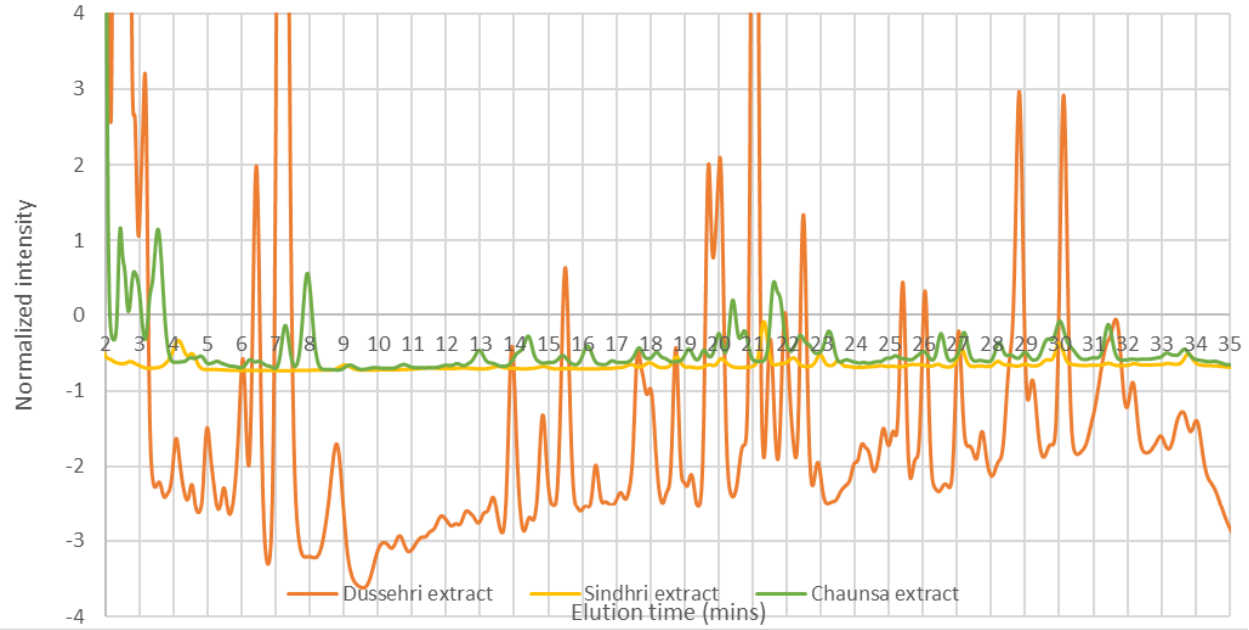

Figure 2: HPLC chromatograms of dussehri, sindhri and chaunsa mango extracts at 260 nm.

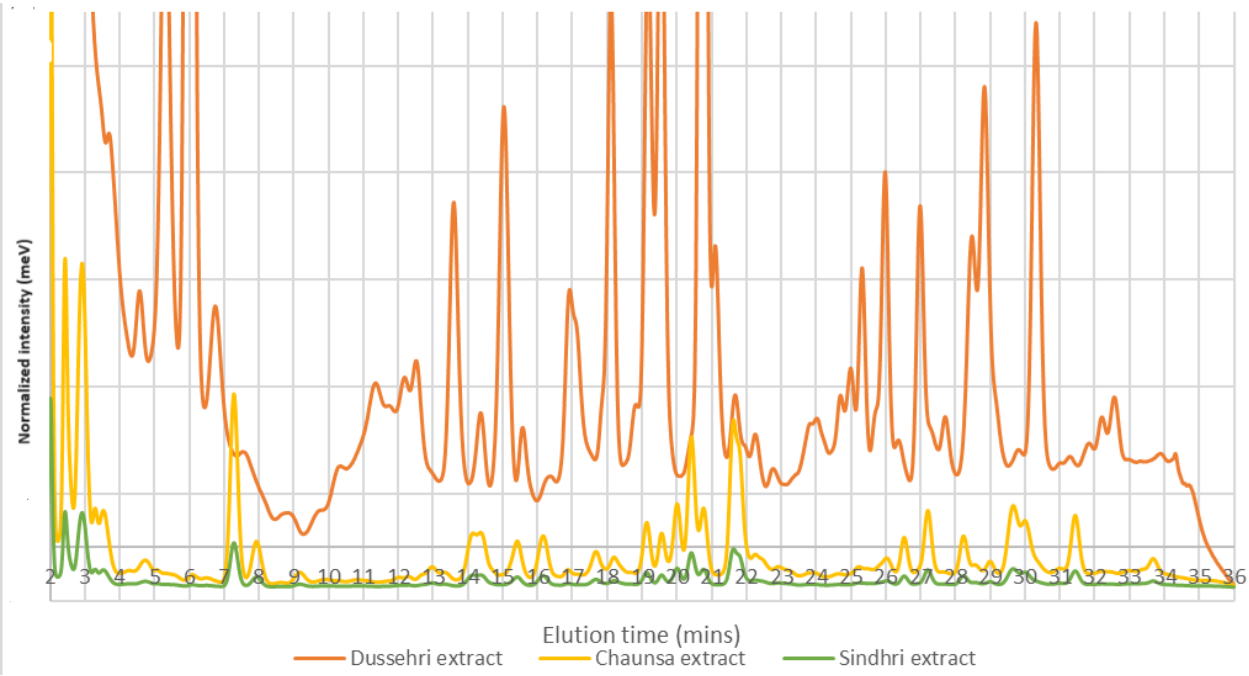

Figure 3: HPLC chromatograms of dussehri, sindhri and chaunsa mango extracts at 280nm.

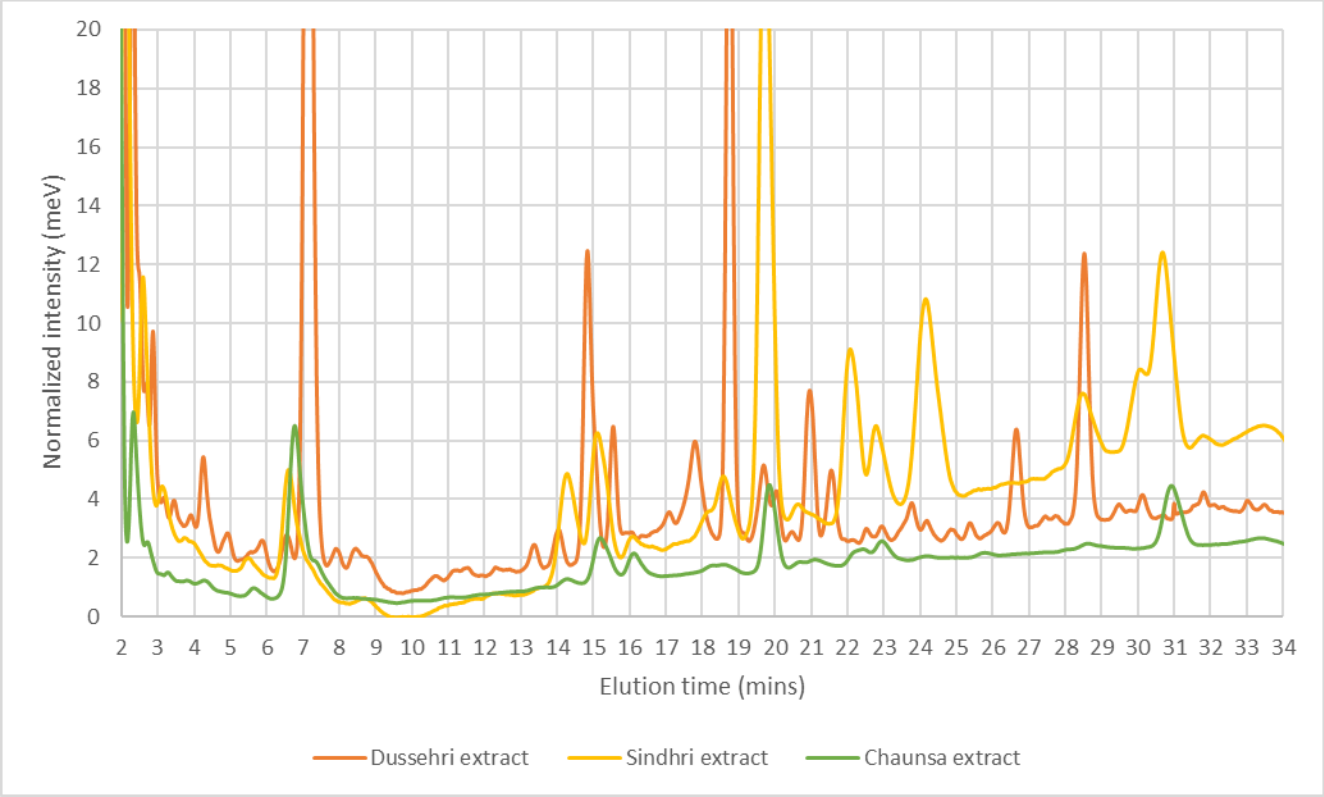

Figure 4: HPLC chromatograms of dussehri, sindhri and chaunsa mango extracts at 320 nm.

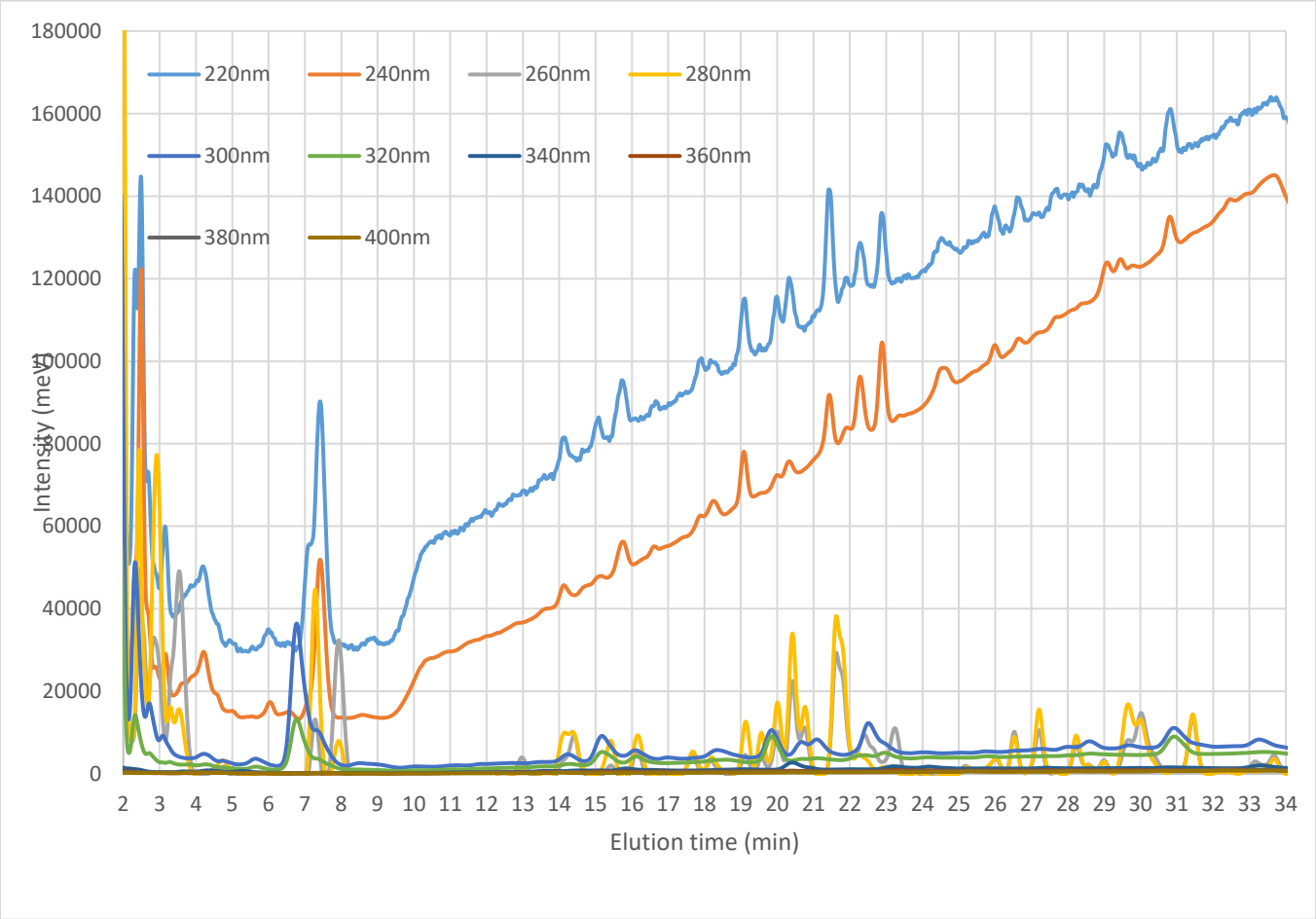

Figure 5: HPLC chromatograms of Dussehri mango extract at various wavelengths.

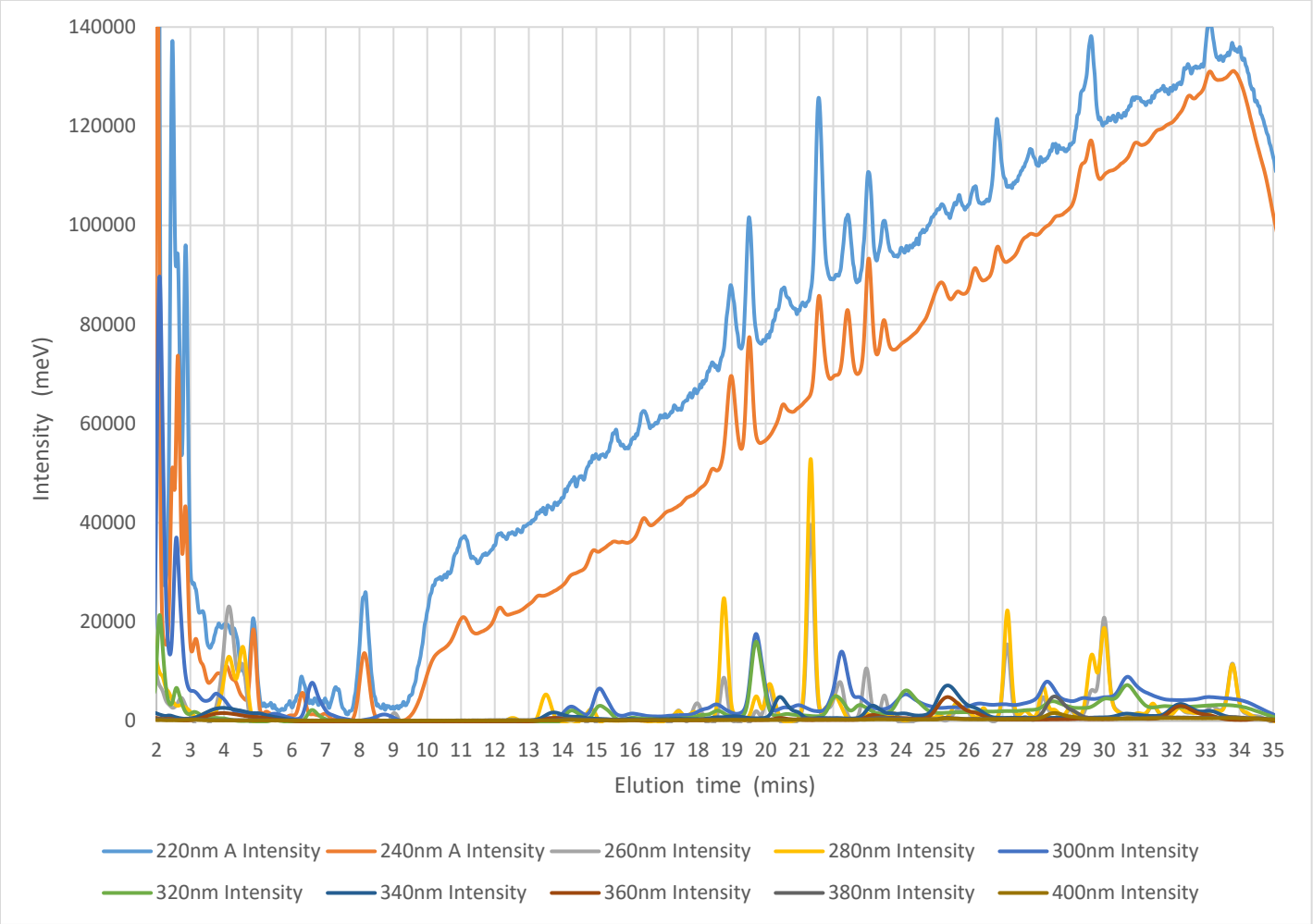

Figure 6: HPLC chromatograms of Sindhri mango extract at various wavelengths.

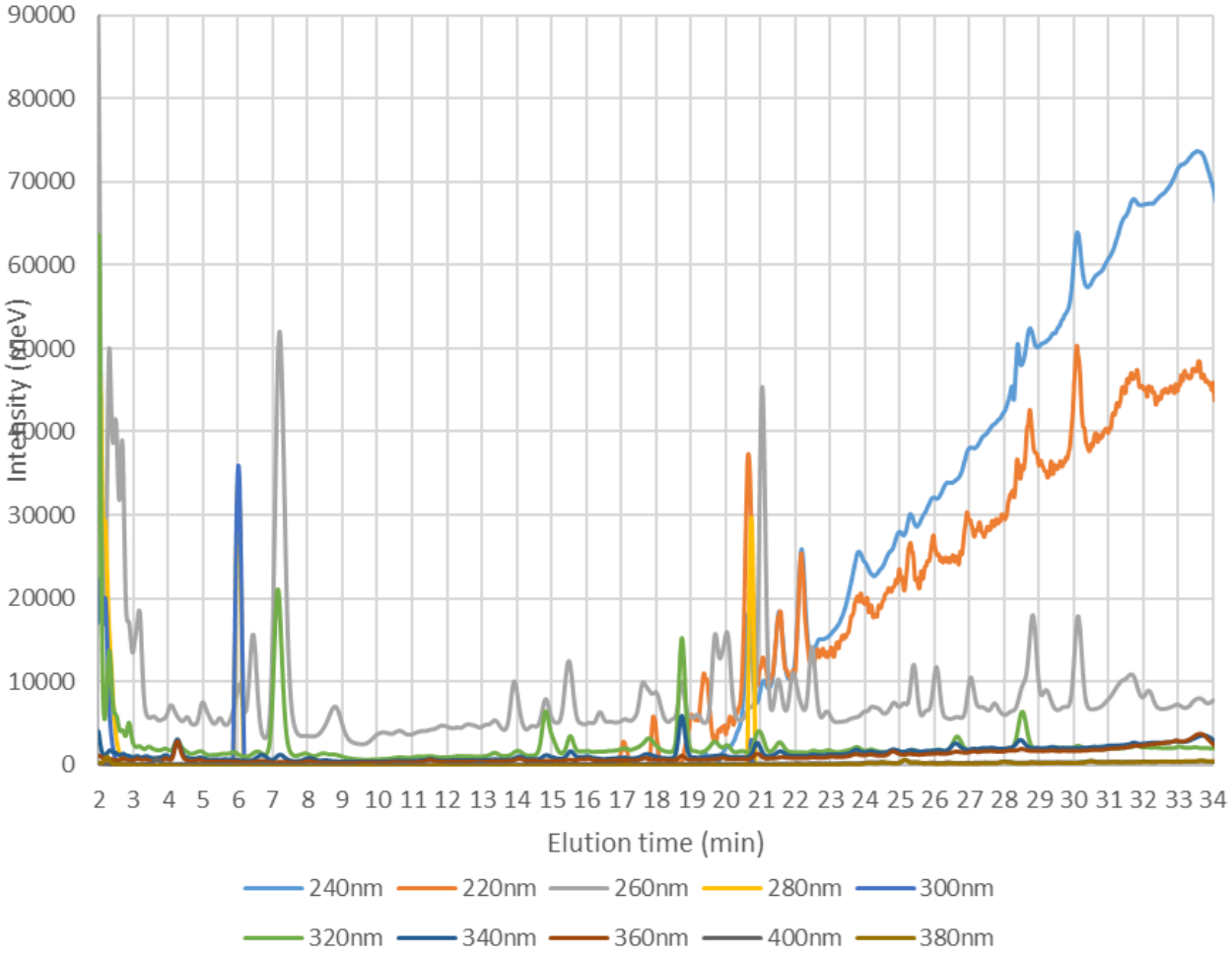

Figure 7: HPLC chromatograms of Chaunsa mango extract at various wavelengths.

DISCUSSION

We have performed a metabolomic analysis of Pakistan's mango varieties using HPLC in the present study. Locally available mango varieties, Dussehri, Sindhri, and Chaunsa were selected for analysis. The mango fruit pulp samples were subjected to maceration for extraction. The solvent system for maceration was a fixed ratio of organic polar solvents for extracting polar pulp compounds, predominantly polyphenols. After maceration, extracts were centrifuged, filtered, and evaporated under pressure to separate unwanted carbohydrates and solvents. Final aqueous extracts of polyphenols were subjected to HPLC analysis. The UV absorption resulted in many peaks in HPLC chromatograms. Chromatograms of mango pulp extracts showed several peaks between time 0-4 minutes. Such peaks presumably represent hydrophilic compounds eluting in void volume as they had little or no binding affinity with the hydrophobic reverse phase C-18 column. The absorbance intensity and number of peaks in chromatograms of extracts decreased significantly with an increase in wavelength (i.e 220 nm to 400 nm). This phenomenon indicates that mango pulp has an abundance of conjugated polyphenols that showed absorption in the UV region of electromagnetic radiation. It has been reported green mango has 45% more flavonoids than mature ripe mango [4]. The variation in flavone absorption among

different extracts can hence be attributed to differences in stages of maturity. Total carotenoid content is a known indicator of ripening, whereas total phenolic content of mango serves to indicate free radical scavenging ability of fruit [5]. Without reference standards of indicative polyphenols such as gallic acid and quercetin and the unavailability of characterization of metabolites by mass spectroscopy, it is hard to deduce inference about the biological value of the mango variety. Chromatographic results of the present study indicated that sindhri mango fruit has a greater amount of polyphenols and can have higher antioxidant capability.

The HPLC data obtained from the present study provides novel insight into Pakistan's mango variety and its characteristic absorption pattern. This data can be utilized to establish variety-specific absorption patterns for developing techniques for the identification of mango variety through artificial intelligence and/or machine learning techniques based on phenolic content. Such an in-silico technique would help evaluate cultivars and enchain harvesting conditions to obtain mangoes of greater antioxidative capacity. It can also help cultivators to program post-harvest handling accordingly to ensure that phenolic content is least disturbed.

Among major mango metabolites, literature reports gallic acid (λmax 220 nm), protocatechuic acid (λmax 240 nm), vanillic acid (λ max 277 nm), ferulic hexoside (λ max 240 nm), hydrolyzable tannins (λ max 280 nm), p-hydroxybenzoic acid (λ max 250 nm) and hydrocinnamic acid (λ max 340 nm) [6].

A comparison of chromatographic data with reported fruit metabolite studies was carried out to comprehend polyphenolic content variation among selected mango varieties.

Dussehri, Chaunsa, and Sindhri extracts at wavelengths 220 nm and 240 nm.

Baseline disturbance was significant for dussehri extract at both 220 and 240 nm whereas for chuansa and sindhri extract same effect was of lesser impact. Such baseline noise issues can be assumed to have arisen due to random errors such as; improper mixing of solutions, decreasing limit of detection or insufficient degassing of solution before run and inadequate column washing that might have allowed residues of the previous sample to remain in common that hindered baseline correction. Chaunsa extract at wavelength 220 nm presented elution of a maximum number of peaks showing the richness of its contents. Sindhri and dussehri extracts have shown considerably less number of peaks at these wavelengths as compared to chaunsa. Among the known polyphenols, gallic acid and quinnic acid or other polyphenols of acidic nature could be found in mango pulp [7].

HPLC of Sindhri extract has resulted in peaks of more phytochemicals at 240nm as compared to chaunsa and dussehri. Metabolites showing absorbance at 240 nm are protoctacheuic acid and hydroxybenzoic acid [7] Hence sindhri extract possesses a higher concentration of acidic polyphenol.

Dussehri, Chaunsa, and Sindhri extracts at wavelengths 260 nm and 280 nm

Chromatograms of all extracts showed well-separated absorption peaks of fine intensity at 260 nm. Polyphenols eluting at 260nm are assumed to be aromatic hydrocarbons. Based on spectral characteristic polyphenols showing absorbance at 260 nm eluting compounds can be vanillic acid, caffeic acid, ferulic acid, and can also be p-hydroxybenzoic acid and its derivatives known to show absorbance at 260nm [8].

At 280 nm, chromatogram of dussehri extract showed more peaks of higher intensity

whereas sindhri and chaunsa chromatograms showed considerably lesser peaks at the same wavelength. Metabolites eluting at 280 nm are catechins, proanthrocynadins, benzoic acid, ellagic acid, ferulic acid, quercetin-3-arabinose, phloretin, and synigic acid [9]. The higher intensity and number of peaks in dussehri extract's chromatogram shows its higher anti-oxidant capacity. Sindhri and chaunsa extract presented peak of gallic acid at 7.85 mins at 280 nm [10] whereas dussehri sample lacked it. This indicates initial ripening stage of dussehri mango where gallic acid is known to be reduced by 22% [11]. The absence of gallic acid also represents lesser antioxidant capacity of dussehri mango fruit

Interestingly at both 260 and 280nm wavelengths dussehri mango chromatogram is significantly different from the other 2 mango varieties. Chromatograms of chaunsa and sindhri extracts at aforementioned wavelengths are superimposable showing property overlay due to similarity in spectral pattern. This distinction in chromatogram makes dessert mango fruit pulp easily distinguishable and recognizable among the other 2 mango varieties at these wavelengths. For a virtual variety recognition program based on polyphenol chromatograms of mango fruit, dussehri mango pulp chromatograms at 260 nm and 280 nm might serve to establish specific identification indicators.

Dussehri, Chaunsa, and Sindhri extracts at wavelengths 300 nm and 320 nm

Dussehri extracts chromatogram at 300 nm presented fewer peaks of low intensity. Sindhri and chaunsa extract chromatograms on the other hand presented peaks of considerable intensity at the same wavelength (data not shown) polyphenols showing absorbance at 300 nm are flavonoids containing carbonyl chromophores [12].

Dusserhi chromatogram has been shown to revive peak intensity at 320 nm. At same wavelength chaunsa extract chromatogram presented comparatively lesser peaks of lower intensity than sindhri and dussehri. Compounds showing this absorbance at 320 nm are phenolic acids other than hydroxyl benzoic acid. Chaunsa extracts chromatogram at 320 nm showed a peak of mangiferin at 31.6 minutes [13]. Lacking mangiferin peak in dussehri and sindhri extracts chromatograms indicates lesser potential antimicrobial and antioxidant capacities. Furthermore, compounds absorbing at 320 nm are cinnamic acid, flavones, and chalcones [14]. It is also important to note that the chromatogram of all three extract varieties showed significant dissimilarity presenting the possibility of easy distinguishability of each variety at these 2 wavelengths based on polyphenolic chromatographic data.

Dussehri, Chaunsa, and Sindhri extracts at wavelengths 340 nm and 360 nm.

Sindhri extracts chromatogram at 340 nm showed a maximum number of peaks with considerable intensity whereas chanusa and dussehri extracts presented fewer peaks at the same wavelength (data not shown). Polyphenols eluting at 340 are flavones and hydrocinnamic acid [15]. At 360nm chaunsa and dussehri chromatograms showed fewer peaks of low intensity as compared to sindhri extract chromatogram. Eluting compounds can tentatively be mycetin, qucertin deoxyhexoside, galactose, flavones, and mangiferin [16].

Interesting the pattern of similarity in chromatograms of sindhri and chaunsa at 260 and 280nm have found to be changed at 340 and 360nm where chromatograms of all extracts showed distinction rather than superimposition. Sindhri and chaunsa extracts hence have polyphenols that are not

excessively aromatic therefore showed greater absorption at these 2 wavelengths.

Dussehri, Chaunsa, and Sindhri extracts at wavelengths 380 nm and 400 nm

Sindhri extract chromatogram showed peaks of higher intensity at 380 nm as compared to chaunsa and dussehri chromatograms. Polyphenols eluting at 380 nm are hydroxyphenyl acetic acid, hydroxyphenyl accetic acids, anthrocynanins, flavones, dihydrochalones, and dihydroflavanoids [17-18].

Baseline noise observed in the chromatogram of all three extracts at 400nm can be attributed to improper buffer mixing and degassing. Sindhri extract chromatogram again at 400 nm showed more peaks reflecting abundance of polyphenols whereas chaunsa and dussehri chromatograms have lacked to exhibit such peak pattern. Polyphenols showing peaks near wavelength 400 nm are flavone, xanthone, and a phenolic acid class of compounds [19-20]. Reports of compound characterization and chromatograms of fruit especially mango are unavailable at 400 nm. So to predict anything without sufficient evidence would be misleading and hence avoided.

CONCLUSION

The present study provided useful information regarding the polyphenolic content of commercial mango varieties grown in Pakistan. The study promotes the use of sensitive and economic RP-HPLC techniques to study plant phytochemical content variations. Despite mango-resource-rich country, the data related to mango fruit metabolomics from Pakistan is scarce. Identification and characterization of fruit metabolites are carried out by mass spectrophotometry, ESI-MS or MS-QTOF and MALDI MS. These spectroscopic instruments are expensive beyond affordability to operate and maintain for most of the medium to low-income countries. We utilized an affordable HPLC technique of unparalleled sensitivity to explore the phytochemical uniqueness of available plant resources. Furthermore, polyphenolic chromatographic variations among mango varieties can also serve as the basis of in-silico mango identification. Chromatographic data of the present study indicated that sindhri mango fruits contain higher amount of polyphenols and can have higher antioxidant capability. Further experiments of metabolite characterization, antioxidant assays, and determination of total phenolic and flavonoid content are required to support this inference.

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