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Probing the Effect of Adulterants on the UV-Visible Absorption Behaviour of Fruit Extracts

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The effect of adulterants on the UV-visible absorption behaviour of pomegranate and orange fruit extracts has been investigated. It was observed that adulterated fruit extracts exhibited significant changes in their peak absorption wavelengths and intensities. The intensity of the characteristic absorption peak of the pomegranate extract was observed to increase almost linearly with the mixing proportion of apple extract. However, the two characteristic absorption peaks of pomegranate exhibited opposite trend: the peak at lower (higher) wavelength side was increased (decreased) on increasing the mixing proportion of apple extract. The orange extract when adulterated by the extract of sweet lime, the change in its UV-visible profile was appeared weaker in comparison to that of pomegranate and apple mixtures. Prominently, the UV-visible absorption-based distinction has been observed to be significantly affected by the proportions of water content present in the fruit extracts. On changing the concentration of water content, remarkable changes in the intensity/peak positions of UV-visible absorption of fruit extracts were observed. The present results would be helpful in the estimation of adulteration in the edible horticultural extracts for quality assessments.

Keywords: UV-visible spectroscopy, Fruit extracts, Adulteration, Dilution.

INTRODUCTION

The fruit juices like that of pomegranate and orange are nutritious choices, each offering distinct health benefits. Among other juices, pomegranate juice excels in antioxidants diversity, while orange juice is a powerhouse of vitamin C and folate [1]. The fruit juices are being consumed by people of all ages around the globe due to their high contents of polyphenols and vitamins [2]. Their increasing demand has led to the risk of adulteration in juices and ensuring their authenticity and quality is a major concern. The cheaper substances like water, sugar solutions, synthetic dyes and some-times, less expensive fruit juices are mixed in the pomegranate juice, compromising their quality and nutritional value. The low-cost juices or added substances are of poor quality and unhygienic practices involves risks of contamination or exposure to undesirable chemicals [1-4]. So, it has been highly appreciable to develop sensitive and quick means to detect/assess the presence of such adulterants in pure extracts.

The UV-visible spectroscopy has been one of the simple and feasible tools to assess the adulterations in fruit juices and it can be coupled with chemometrics to discriminate the adulteration more precisely [5]. Numerous methodologies such as mass spectroscopy (MS), nuclear magnetic resonance (NMR) *etc.* could too be utilised to estimate the adulterations in fruit juices. The mass spectroscopy in combination with multivariate statistical analysis could be effectively employed to detect adulteration in fruit juices like that of pomegranate [1,6,7]. The NMR based analysis assisted by chemometrics could be applied to assess the proportions of pure fruit juices in blends of apples, orange, pineapple and pomegranate juices and adulteration in grape nectars [8-10]. In a study, an efficient method utilizing 1D and 2D NMR spectroscopy (including NaOH sequences and spiking with model compounds) resulted efficient quantification of various metabolites like sugars, organic acids and amino acids [8-10].

Researchers demonstrated the utility of $MnCl_2$ as a relaxation agent and confirmed the robustness of quantitative QEC-HSQC experiments. This comprehensive study provided proof-of-concept for 1D and 2D NMR methods in targeted and untargeted analysis of pomegranate juice, with potential for broader application to complex matrices and differentiation

between cultivars and adulteration [10-12]. Moreover, NMR spectroscopy has been established as a robust method for rapidly analysing mixtures at the molecular level in food science without requiring their separation or purification. The NMR based profiling combined with chemometrics such as partial least squares (PLS) regression, classification rules, Pareto scaling and covariance selection approaches *etc*. could be opted to discriminate the authentic and adulterated orange juices [11-13]. Despite its potential, it remains underutilised due to high cost, low sensitivity and lack of expertise [12,13].

In a recent report, the application of several spectroscopic techniques (NIR, FTIR, HSI, Raman, UV-vis and FS) in detecting the adulteration in horticulture products has been compared for powdered food, meat, honey, drink, edible oil and dairy product [14]. Shafiee & Minaei [15] analysed the concentrations of 22 trace elements in a large set of Australian and Brazilian orange juices and peel extracts with inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and ICP-MS, revealing the distinct elemental profiles that enabled differentiation of samples according to their origin and product type. Mohammadian *et al.* [16] have utilised Fourier transform infrared (FTIR) spectroscopy in combination with various pattern recognition techniques like principal component analysis (PCA), variable importance in projection and PLS discriminant analysis and counter propagation artificial neural network to identify fraud in lime juice, classifying samples into natural and reconstructed categories [16]. The PCA combined with FTIR spectroscopy was used to determine the adulteration in pomegranate juice mixed with grape and apple juices or simply diluted with water [1]. Similarly, others researchers too [17,18] reported that the pomegranate adulteration with these fillers can be detected by UV-visible spectroscopy and unsupervised PCA analysis.

The HPLC photodiode array method was employed by Ooghe *et al.* [19] to detect the orange juice adulteration by analysing flavanone glycoside patterns. The HPLC fingerprinting, when combined with mathematical processing (chemometrics) could serve as a powerful and versatile analytical strategy for various food-related applications [20-22]. Such studies disclosed the authenticity of pomegranate beverages by identifying red grape-derived components and revealed that many commercial 'pomegranate' products are the adulterated mixtures. Similarly, the combination of high-resolution ultra-performance liquid chromatography (UPLC) with quadrupole time-of-flight mass spectrometry (QToF-MS) provides sensitive mass analysis and unique chemical fingerprints for sample authentication, enabling effective detection of fruit juice adulteration, even at low levels, through precise separation and identification of complex mixtures [23,24].

Kundu *et al.* [24] have developed a highly sensitive electrochemical biosensor by utilizing a carbon nanotubes-Fe_3O_4 nanocomposite for the rapid and selective detection of formaldehyde adulteration in orange juice. There are some other methods to detect adulteration like constant phase element (CPE) impedance-based capacitive sensor combined with PCA and linear discriminant analysis (LDA) for grape fruit juice adulteration [25]. A recent investigation explored various e-nose techniques, emphasizing the use of chemometric analysis with gas sensor arrays, which has shown significant promise in ensuring food quality and detecting fraud [26]. The contributions of sensor-based e-nose systems are extensively examined to provide a comprehensive overview of their role in addressing food adulteration [26]. The compounds/mixtures having free radicals or transition metal ions could be detected by using a non-destructive electron paramagnetic resonance (EPR) spectroscopy. The EPR could accurately determine the Trolox equivalent antioxidant capacity (TEAC) values to provide authentication of samples with lower errors and it is not affected by the colour or turbidity of the samples [27].

The UV-visble spectroscopy and one-class classifiers have been used to authenticate honey and detect adulteration with various sugar syrups, highlighting its ability to prevent fraudulent labelling with minimal sample preparation [28]. The machine learning combined with near-infrared spectroscopy could be utilised for the rapid and precise detection and quantification of adulterants in various fruit juices [29]. Włodarska *et al.* [30] have compared ultraviolet, visible and near-infrared spectroscopy (combined with chemometrics) as rapid, non-destructive assessment of apple juice quality parameters and identified the optimal methods for specific attributes. Recently, the performances of UV-visible and FTIR spectroscopies combined with chemometric methods was investigated to determine adulteration of pomegranate juice with dark coloured sour cherry and black carrot juices [31].

Among the various techniques used to detect adulteration and assess the quality of horticultural extracts, UV-visible spectroscopy is a powerful and reliable analytical method that measures light absorbance or transmittance as a function of wavelength. The unique spectral signatures of different components in liquid food matrices, such as fruit juices, enable the identification and quantification of adulterants, while the absorption profiles of constituent compounds are sensitive to factors such as pH, storage conditions and dilution. Consequently, deviations or modifications in characteristic absorption patterns arising from interactions between UV-visible–active compounds and adulterant species can be effectively used to distinguish fresh, stored, pure and adulterated fruit extracts [32]. The present report aims to investigate the feasibility of using UV-visible spectroscopy for the detection of adulteration: (i) juice into juice adulteration (apple into pomegranate and sweet lime into orange, (ii) dilution of juices (pomegranate, apple, orange and sweet lime) by distilled water. The freshly prepared fruit extracts were deliberately adulterated and their UV-visible absorption spectra is analysed to assess its suitability as a methodology for the qualitative identification of adulteration in fruit extracts. The findings of this investigation are an effort for the development of effective quality control measures to ensure the authenticity and safety of fruit juice products for consumers.

EXPERIMENTAL

Sample preparation: The fresh fruits of pomegranates, apples, oranges and sweet limes were purchased from local fruits vendors. The same variety of fruits were used to extract the samples for all the measurements. The fruits were peeled and extracted using hand-squeezer. The fruit extracts were then filtered using Whatman filter paper (grad 1) having pore

size 11 μm. The filtered samples were centrifuged at 1500 rpm for 10 min to separate the clear extracts which were then used for recording UV-visible absorption spectra. All the measurements and sample preparations were carried out at room temperature (30 ºC). For adulterated or diluted samples, the mixtures were shaken well for proper mixing and kept for 5 min and then their UV-visible spectra were recorded. For UV-visible spectral profile, the glass cuvettes were used and 4 mL volume of the samples (pure and adulterated fruit extracts) was filled in the cuvettes. Another similar glass cuvette filled with the distilled water was used for the reference sample. The UV-visible absorption profiles were recorded in the wavelength range 350 nm to 600 nm using microprocessor controlled double beam UV-visible spectrophotometer, Lasany LI-2704 with 1 nm resolution. The FTIR transmittance profile of pure and mixed extracts were recorded using FTIR spectrometer, Tensor 37, Bruker optics.

RESULTS AND DISCUSSION

To observe the effect on the UV-visible absorption profiles of pomegranate extract due to addition of apple juice, the UV-visible spectra of pure pomegranate, pure apple and apple mixed pomegranate juices samples were recorded in the wavelength range of 350 nm to 600 nm. Fig. 1 illustrates the distinct UV-visible absorption profiles of freshly prepared extracts of apple, pomegranate and their mixtures in different proportions (volume/volume). The absorption profile of pure apple extract displayed a broad absorption band with peak absorptions around 425 nm, 500 nm and a weak peak at ~545 nm (Fig. 1a). Such spectral characteristics are typical for fruit juices and are influenced by the presence of various bioactive compounds. The UV absorption profile of pomegranate extract exhibited two broad bands observed at 395 nm and 518 nm (Fig. 1b). The bands in the UV-visible spectra correspond to different chromophores present in fruit extracts such as nitro groups, carbonyl groups, double and triple bonds, conjugated double bonds, *etc.* and the pigments conjugated double bonds present in the fruit extracts could, too, cause prominent absorption in the visible range [30].

The absorption peaks near 500 nm in apple as well as pomegranate extracts could be due to the presence of anthocyanin derivatives and the position of the absorption peak could vary depending on the structures of the derivatives [1,3,33]. Recently, the UV-visible absorption profile of anthocyanin extracted from different species of berries and plums was recorded and a dominant absorption maximum was observed around 500 nm along with two weak peaks at 310 nm and 415 nm by Ionescu *et al*. [34]. They also observed the significant decrease in the intensity of absorption on increasing the pH from 1 to 4.5, however, no shift in the peak position was observed on changing the pH of the solution. The positions of the absorption peaks of fruit juices could vary depending on their varieties, origin and freshness. The water/moisture content in a freshly harvested fruit is greater and it significantly decreases for stored fruits. The water content could remarkably modify the UV-visible behaviour of the fruit extracts and the same has been comprehensively analysed and discussed in the later half. It is clear from Fig. 1b that the absorption bands of pomegranate are comparatively sharper and well resolved than that appeared for apple extract. These unique spectra of pure apple and pomegranate extracts serve as crucial baselines for identifying their presence in mixtures, as highlighted in the studies using UV-visible spectroscopy for juice analysis [20,35]. To analyse the UV-visible absorption behaviour of the pomegranate extract on adulterating by apple extract, the UV-visible absorption of freshly prepared mixtures of these extracts were recorded. Fig. 1c shows the UV-visible profiles of pomegranate extract mixed uniformly with different proportions (5-40% v/v) in the wavelength range 350-600 nm. The presence of the apple extract has been clearly reflected in the UV-visible profile of the mixtures in terms of modified intensity as well as the position of the absorption peaks.

The intensities of the absorption bands at 395 nm and 518 nm (pure pomegranate) have been increased remarkably with the mixing concentration of apple extract (Fig. 2). The intensity of 395 nm absorption peak has been increased from 2 A.U. to 2.55 A.U. exhibiting almost linear variation with the mixing concentrations of apple extract (Fig. 2a). The similar behaviour of intensity of the absorption peak at 518 nm was observed indicating the linear response of intensity with the mixing concentration of apple into the pomegranate extract. The contribution of the absorption from apple could have resulted the increase in the effective intensity of the mixture. On increasing the concentration of apple extract in the mixture, the intensity of the absorption peaks started approaching the intensity value corresponding to that of pure apple and attained the same at higher concentrations (> 40% v/v).

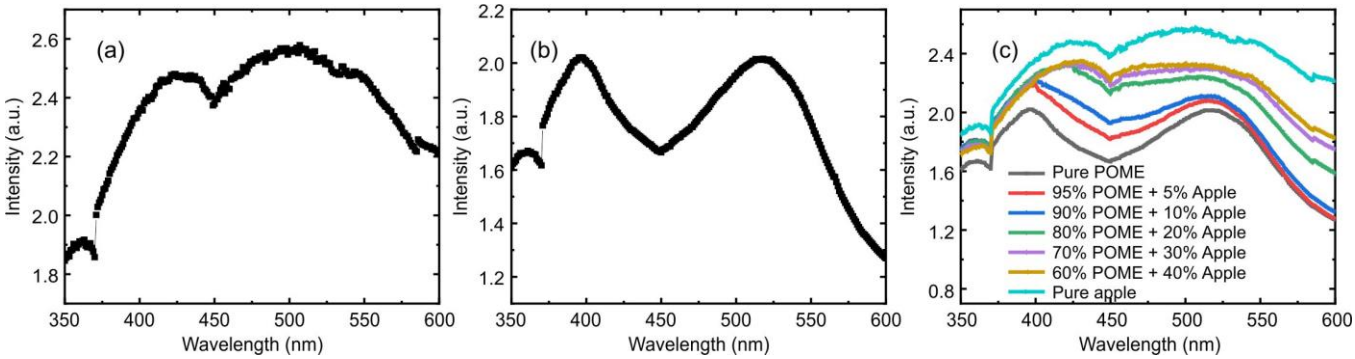

Fig. 1. UV-visible absorption profiles of freshly prepared extracts of (a) pure apple, (b) pure pomegranate and (c) pomegranate extract mixed with apple extract in different proportions

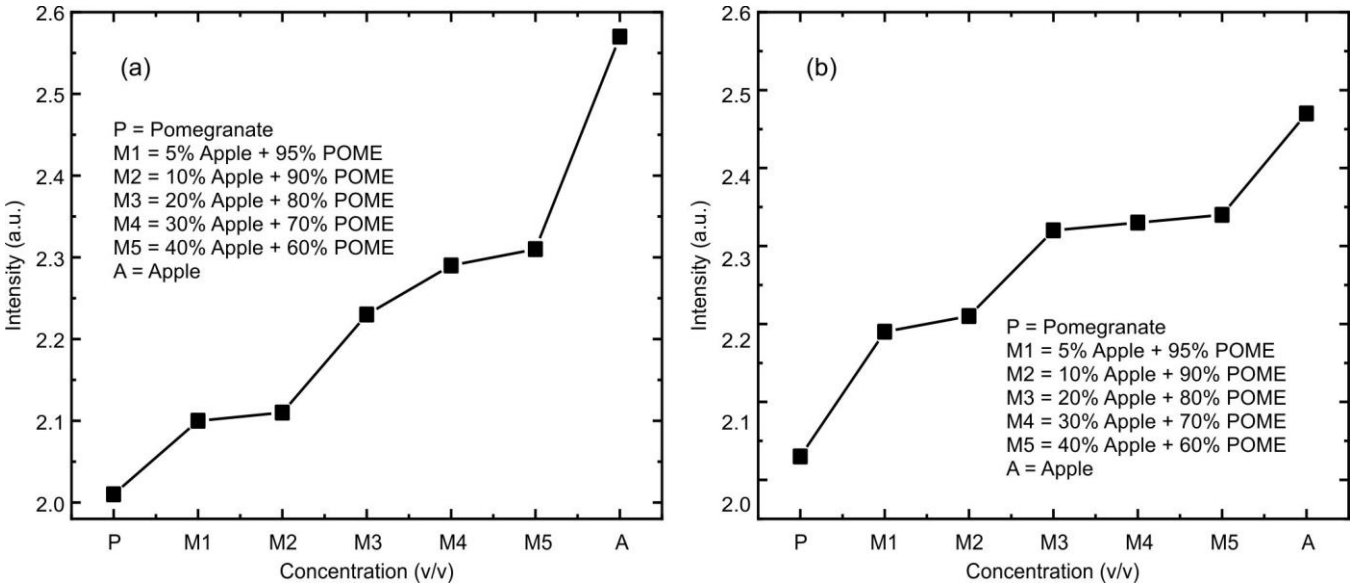

Fig. 2. Variation of UV-visible absorption intensities of absorption peaks at (a) 395 nm and (b) 518 nm of pomegranate extract mixed with apple extract in different proportions (M1 = 5% v/v, M2 = 10% v/v, M3 = 20% v/v, M4 = 30% v/v and M5 = 40% v/v)

However, it is interesting to note that the wavelength of the absorption peak, too, has been shifted on increasing the concentration of apple extract. Fig. 3 shows the variation of the wave-lengths of absorption peak of pomegranate extract with the mixing concentration of apple extract. It can be observed that as the apple extract concentration increases, the peak wavelength got red shifted from ~395 nm (for pure pomegranate) to around 430 nm for the mixture with 40% apple extract. This trend suggested that the presence of apple extract influenced the absorption characteristics of the pomegranate component, potentially due to interactions between compounds from the two fruits. The consistent shift in this low wavelength peak (~395 nm) highlighted the impact of adulteration (juice into juice) on the spectral properties of the individual components. The distinct peak characterizing the pomegranate extract at ~395 nm began to broaden and shifted towards higher wavelengths with increasing apple content. The absorption characteristics of apple extracts, particularly the broader band at higher wavelength started to emerge and became more pronounced as the apple concentration increased. The increase in the intensity of absorption of mixture could be understood by considering the Beer-Lambert law stating that the total absorbance of a mixture is the sum of the absorbance of its individual components [35]. The mixing of the apple extract caused the shifting of the peak positions towards red as well as blue side (Fig. 3).

The absorption peak of pure pomegranate was blue shifted when adulterated with apple extract *i.e.*, the peak around 518 nm (pure pomegranate) was shifted linearly to ~507 nm on increasing the mixing concentration of apple from 5 to 40% v/v (Fig. 3b). However, the peak of pomegranate extract at 395 nm was red shifted from ~395 nm to 430 nm when the proportion of apple extract was increased from 5 to 40% v/v (Fig. 3a). The intensity of absorption and shifting of the absorption peak

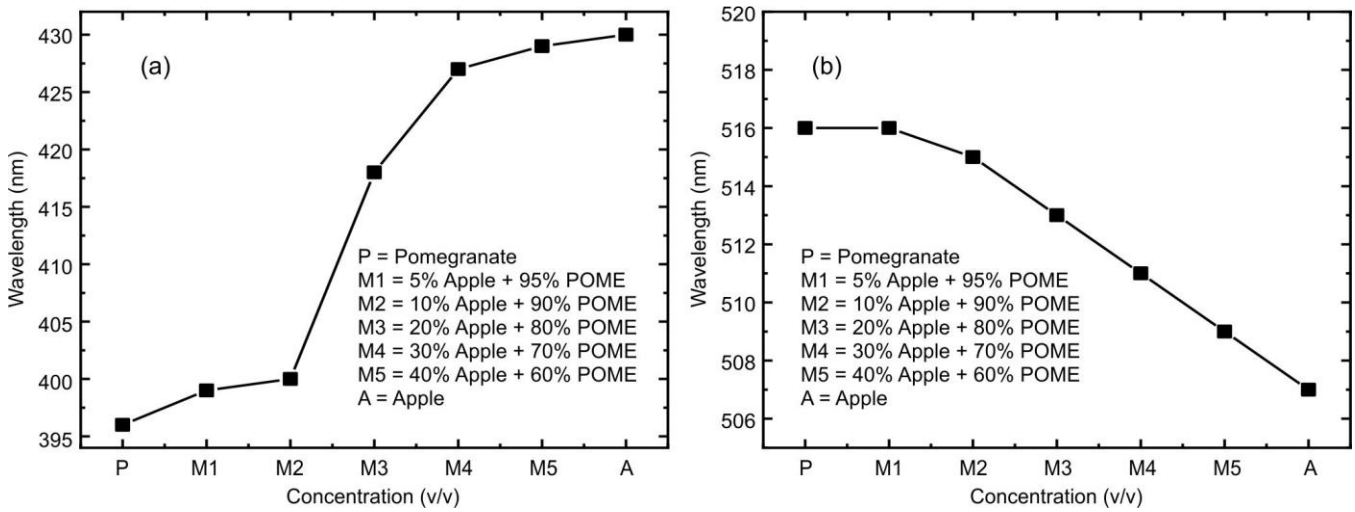

Fig. 3. Variation of peak wavelengths at (a) 395 nm and (b) 518 nm of pomegranate extract mixed with apple extracts (M1 = 5% v/v, M2 = 10% v/v, M3 = 20% v/v, M4 = 30% v/v and M5 = 40% v/v) in different concentrations

is attributable due to the changed environment of the chromophores on mixing of the apple extract [36]. The intensities of absorption and the peak absorption wavelength could critically depend on the concentration of the solution surrounding the chromophores and the variations in the intensities/wavelengths could exhibit a linear response [36]. Such kind of linearity could offer the assessment of the adulterations made in the horticultural extracts. The pomegranate and apple are the two different species and hence showed distinct UV-visible profiles easily distinguishable in terms of their intensity and peak absorption peaks.

To analyze the UV-visible absorption behaviour of fruit extract of same species, we have recorded the UV-visible absorption spectra of freshly extracted orange and sweet lime extracts in their pure form as well as their mixtures (Fig. 4). It is observed that both the extracts (pure orange and sweet lime) exhibited almost same absorption profiles showing a broad absorption band with relatively comparable intensities in the wavelength range 350-370 nm peaking at ~360 nm (Fig. 4a). This absorption band could be attributed as a signature of flavonoid rutin exhibiting one of its characteristic absorptions at 358 nm [37,38]. The orange extract when mixed (in different proportions) with sweet lime extract, the corresponding absorption profiles appeared almost overlapped spectrally with a slight variation in the intensity of peak absorption. Fig. 4b depicts the variation of intensity of peak absorption of orange extract mixed with sweet lime extract in different concentrations. The intensity of peak absorption is visually observed to decrease on increasing the concentration of sweet lime extract and interestingly, the variation in intensity appeared as linear function of the concentration of sweet lime added. However, no remarkable shift in the absorption band was observed as the wavelength corresponding to the peak absorption appeared to be independent of the concentration of sweet lime added (Fig. 4c). It has been observed that the absorption took place in these kinds of citrus juices due to presence of vitamin C, carotenoids, phenolics, *etc*. among which the phenolics are the primary contributors, while vitamin C offers a moderate contribution and carotenoids provide a negligible effect [39-41].

The spectral overlap between orange and sweet lime makes it challenging to distinguish between the two using their UV-visible profiles. He *et al.* [42] reported the spectral similarity among citrus fruits that can make their purity assessment complicate. The NIR spectroscopy and data mining could be utilised to assess the purity of lime juice with good performance in distinguishing natural samples from that of the synthetic samples [15]. There are other reports that indicated the necessity of a distinct spectral marker or shift in absorption maxima to distinguish the low-level adulteration in fruit juices by using UV-visible spectroscopy technique [43,44]. Haque *et al.* [45] too suggested that the adulteration detection *via* UV-visible absorption could become challenging when adulterant has similar chromophores as the original sample. Moreover, analytical noise, natural variation in fruit composition and aging of samples could influence UV-visible profiles and hence it becomes crucial to be considered carefully while analyzing the data [46,47].

The addition of one extract into the other could change the proportions of water in the mixtures and, also the proportions of water will be different in fresh and stored fruits extracts. In view of this fact, the effect of water content (dilution) on the UV-visible absorption behaviour of fresh fruit extracts was investigated by diluting them with different concentrations of distilled water. Fig. 5 shows the UV-visible absorption profiles, variation in intensity and shift in the spectral peak of apple extract diluted by distilled water. It is clear that the UV-visible signature of apple extract has been significantly modified due to the presence of distilled water. The intensity of absorption has been decreased for all the peaks at 425 nm, 500 nm and 545 nm. This decrease in the intensity appeared almost linear with the distilled water content that can be clearly seen from Fig. 5b. The intensity of absorption peak at 500 nm is decreased from ~2.55 A.U. to ~2.30 A.U. in almost a linear fashion. The decrease in the absorption intensity could be understood by the fact that on adding the distilled water, the density of the chromophore molecules gets decreased to give reduced absorption and is consistent with Beer-Lambert's law [35]. Interestingly, the peak wavelength of the absorption band at 500 nm exhibited a remarkable blue shift on increasing the concentration of distilled water (Fig. 5c). The absorption peak of apple extract was shifted from 500 nm to 465 nm when the mixing concentration of distilled water was increased from 10 to 50% (v/v).

It is, however, noticeable that the shifting in the peak position was observed for peak at 500 nm only, the remaining

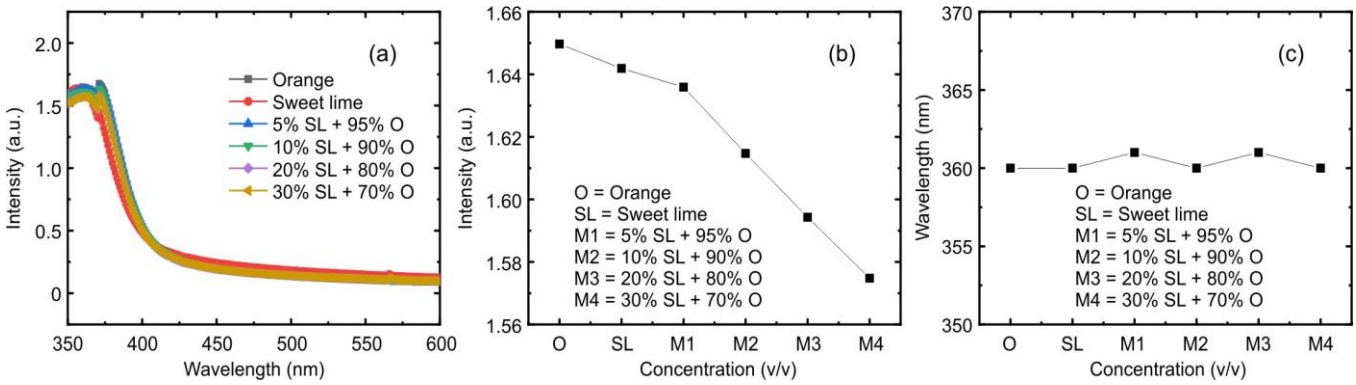

Fig. 4. UV-visible absorption spectra of (a) freshly extracted orange (O), sweet lime (SL) and orange extract mixed with that of sweet lime in different proportions (M1 = 5% v/v, M2 = 10% v/v, M3 = 20% v/v, M4 = 30% v/v), (b) and (c) Variation of intensity and wavelengths of the peak absorptions of orange extract on mixing with sweet lime, respectively

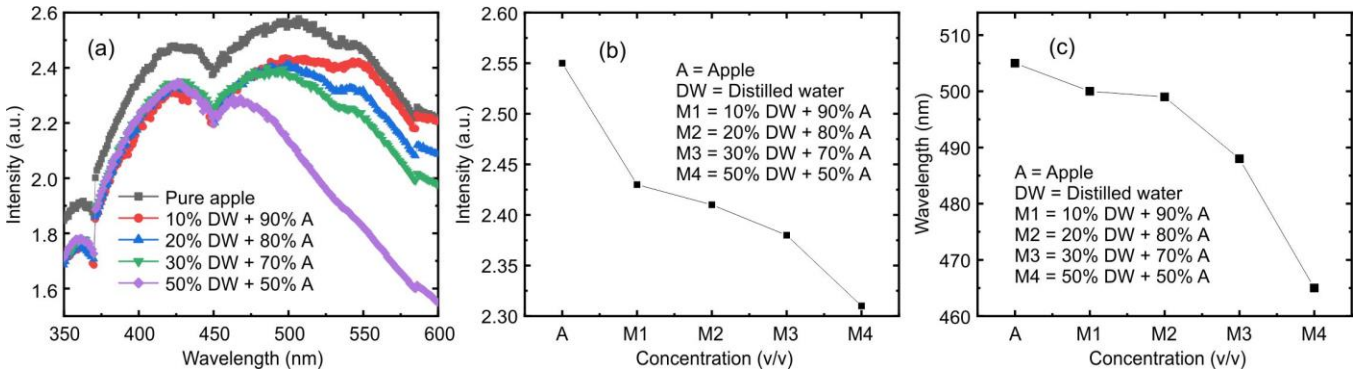

Fig. 5. UV-visible absorption profiles of (a) pure apple (A) extract and diluted by distilled water (DW) in different proportions (M1 = 10% v/v, M2 = 20% v/v, M3 = 30% v/v, M4 = 50% v/v). (b) and (c) Variation of peak absorption intensity and peak wavelength, respectively of apple extract on dilution with distilled water (DW)

two peaks at 425 nm and 545 nm were found independent to the added water content. Fig. 6 shows the UV-visible absorption spectra of pomegranate extract diluted with different concentrations of distilled water. Like that of apple extract, significant change in the intensity and peak position of pomegranate extract is appeared on diluting with distilled water. The absorption intensity of peak at 518 nm exhibited significant decrease from ~2 A.U. to ~1.20 A.U., whereas the peak intensity of peak at 395 nm decreased from 2 A.U. to 1.75 A.U. (Fig. 6b). Moreover, the addition of distilled water caused remarkable shifting of the peak positions too. The wavelengths of ~395 nm and 518 nm were blue shifted to ~384 nm and ~511 nm, respectively (Fig. 6c).

Fig. 7 shows the effect of water addition on the UV-visible spectra of sweet lime extract. Clearly, the dilution affected the intensity and peak position of sweet lime extract a similar fashion as observed for distilled water diluted apple and pomegranate extracts. The intensity of peak absorption was decreased from 1.65 A.U. to 1.45 A.U. whereas the peak wavelength was blue shifted from 360 nm to 347 nm (Fig. 7b-c). It is worth to mention that the behaviours of intensity and peak wavelength of fruit extracts mixed with distilled water

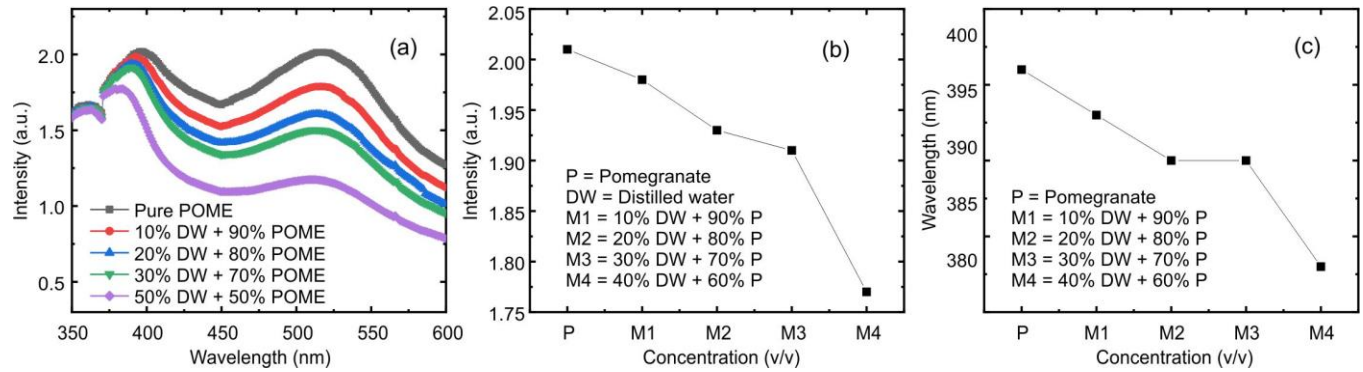

Fig. 6. UV-visible absorption profiles of (a) pure pomegranate extract and pomegranate extract diluted by distilled water (DW) in different proportions (M1 = 10% v/v, M2 = 20% v/v, M3 = 30% v/v, M4 = 50% v/v), (b) and (c) Variation of peak absorption intensity and peak wavelength, respectively of pomegranate extract on dilution with DW

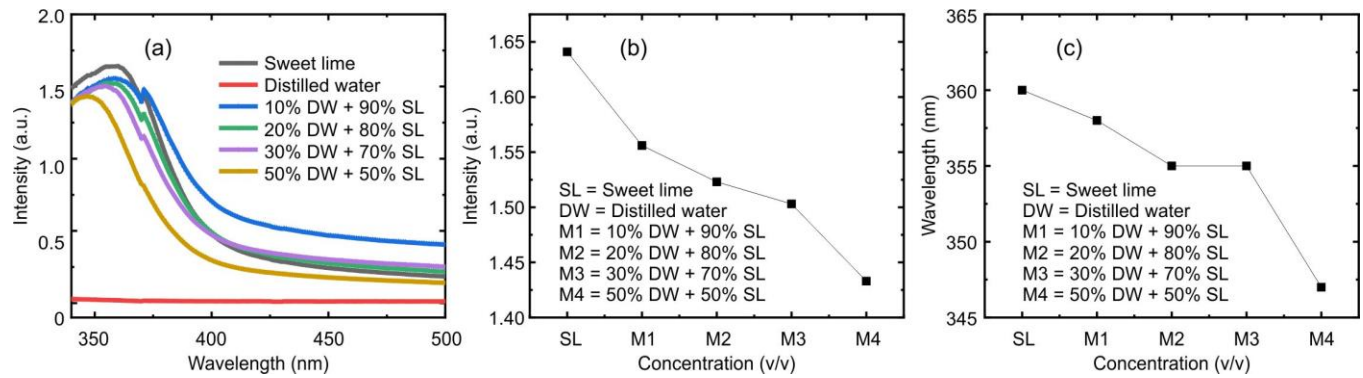

Fig. 7. UV-visible absorption profiles of (a) pure sweet lime (SL) and SL diluted by distilled water (DW) in different proportions (M1 = 10% v/v, M2 = 20% v/v, M3 = 30% v/v, M4 = 50% v/v), (b) and (c) Variation of peak absorption intensity and peak wavelength, respectively of SL extract on dilution with DW

appeared quite different in comparison to their behaviours when the fruits were adulterated by other fruits. In case of dilution with distilled water, the peak absorption wavelength exhibited blue whereas it exhibited the red shift when adulterated with fruit extracts. The variation in the intensity and peak absorption wavelength of extracts of pomegranate and sweet lime exhibited the same trend as that of apple extracts on diluting with distilled water.

As the variation in the intensity and shift in peak wavelength is observed as almost a linear function of distilled water concentration, the results may be utilised to assess the proportion of water content in juices. The stored/aged fruits will contain less content of water and hence the freshness of the fruits/fruit juices could also be assessed by analyzing their UV-visible absorption profiles. The shift in the absorption band on changing the concentration of aqueous solutions of certain chemicals was studied by Tong *et al.* [36] and noticed a remarkable linear shifting of the UV peak absorption towards red. This red shift of UV bands is due to the concentration dependent energy produced by electronic transitions. In present study of diluted fruit extracts, the shifting of absorption band towards blue could be attributed due to the increased energy of electronic transitions on diluting the extracts. The observance of variation in the peak intensity and wavelengths of fruit extracts on diluting them with water is of great importance. The chromophores such as anthocyanin, flavonoids, *etc.* responsible for the characteristic UV-visible signatures of fruit extracts could be affected greatly due to presence of different proportions of water/moisture and hence this becomes one of the crucial parameters to be considered seriously while assessing the adulterations in the horticultural extracts containing water. In case of mixing of two fruit extracts or diluting the fruit extracts with distilled water caused the change in the pH values that, in turn, has reflected in terms of modified absorption intensity and shift in the peak wavelengths. In both cases, the pH values of the samples were found to be changed and the same has been illustrated in Fig. 8. The pH value of pomegranate extract was observed to slightly increased (became basic) when the mixing proportions of apple extract was increased. Similarly, dilution of extracts with distilled water, too, resulted in terms of increased pH values of diluted fruit extracts (Fig. 8). As can be seen from Fig. 8a that the pH value of the pomegranate/apple mixture is monotonically increased with the mixing concentration of apple extract. However, the pH value of mixtures of orange and sweet lime extracts was found to change very slightly (almost independent) as both belongs to the same kind of citrus fruits. The pH values of pomegranate and sweet lime were observed almost independent of the distilled water dilution whereas, the pH values of apple extract were slightly increased on dilution (Fig. 8b). The chromophores such as anthocyanin, flavonoids, *etc.* responsible for the characteristic absorption in the UV region could be affected by their protonation/de protonation on conjugation with water [48].

As discussed earlier, the FTIR spectroscopy coupled with chemometrics could offer a rapid, high-throughput and quantitative method utilizing to detect and quantify orange juice adulteration, particularly dilution disguised with sugars [49]. The intensities of transmittance of different IR active bands could reflect a systematic variation on changing the surrounding environment of molecular bonds. To make a comparative assessment on the qualitative detection of adulteration of pomegranate juice with that of apple, the FTIR profile of pure pomegranate and its mixtures with apple extract were recorded (Fig. 9). The fingerprint bands of pure and mixed pomegranate extracts exhibited a slight variation in the coefficient of transmittance, however, broadly they have almost overlapped spectrally on each other indicating the contributions from same IR active bands of pomegranate and apple extracts.

The IR band appearing near 1065 cm^{-1} is found to reflect a remarkable distinction for different mixing concentrations of apple extract in pomegranate one. This IR band could arise due to stretching of C–O or bending of free hydroxyl group OH [18,50]. The IR transmittance is observed to vary linearly from 0.925% to 0.905% when the mixing concentration of apple extract (in pomegranate) was increased from 0 to 40% v/v (Fig. 9b-c). As no remarkable spectral change was observed in the FTIR transmittance profile of pomegranate/apple mixture extract, relying on intensity variations is quite challenging for the purpose of adulteration detection. Such variation in the

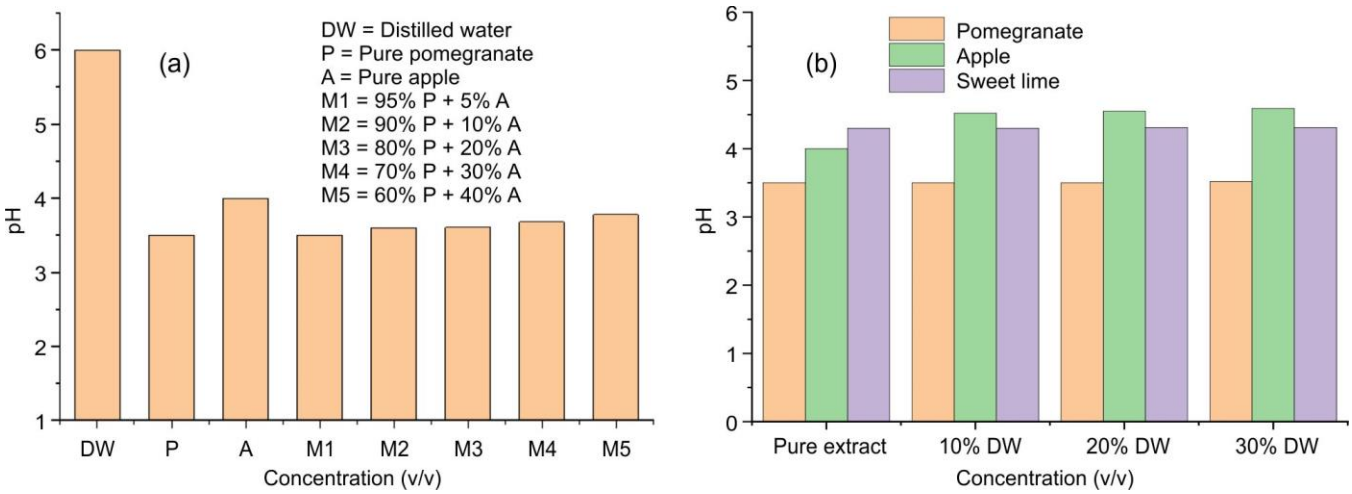

Fig. 8. Variation of pH values of (a) pomegranate extract on mixing with apple extract and (b) pomegranate, apple and sweet lime extracts on diluting with distilled water (DW)

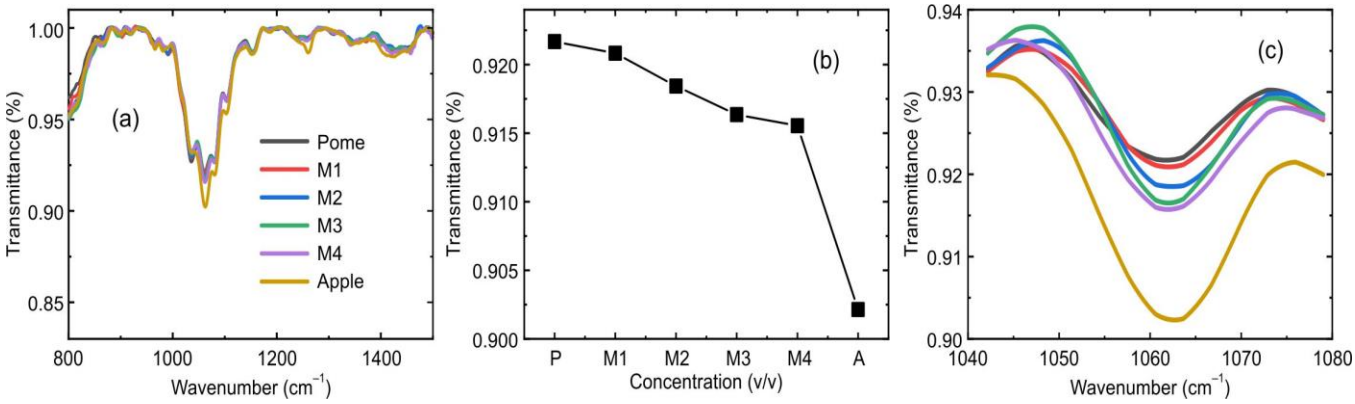

Fig. 9. (a) FTIR profile of pure pomegranate (Pome) extract and mixed with that of apple in different proportions (M1 = 10% v/v, M2 = 20% v/v, M3 = 30% v/v and M4 = 50% v/v) at RT; (b) the variation of the IR transmittance intensity with the mixing concentration of apple into the pomegranate extract; (c) the enlarged view of the peak transmittance ~1063 cm^{-1} of mixture

transmittance, however weak, may be calibrated and coupled with computational means to estimate the adulterations in the fruit juices specially for juice-in-juice adulterations.

Conclusion

The UV-visible absorption spectra of various fruit extracts have been analysed in their pure form, mixing them with other fruit extracts and distilled water in the wavelength range 350 nm to 600 nm. The UV-visible absorption spectra of pure and adulterated samples (juice into juice) have exhibited remarkable linear variations in terms of peak absorption intensities and peak absorption wavelengths. Different proportions (v/v) of apple/sweet lime extract were mixed with pomegranate/orange extracts, respectively to compare the intensity/peak wavelengths with their pure counter parts. Significant changes (in intensity as well as peak wavelengths) have been observed in the UV-visible absorption behaviour of fruit extract when adulterated with other juices. The effect of dilution (with distilled water) on the UV-visible profiles of fruit extracts has also been analysed and the observed intensities/peak positions were found to exhibit linear variations with the concentration of distilled water. The effect of adulteration on the infrared signatures of fruit extracts has also been analysed using FTIR spectroscopy and the behaviours were compared with that of UV-visible profiles. The present results would be helpful in the estimation of adulteration and freshness of horticultural extracts for quality assessments.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

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