



Effect of in-vitro digestion on the bio active compounds and biological activities of fruit pomaces

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Abstract The effect of gastro intestinal digestion on total phenolic contents (TPC), total flavonoid contents (TFC), radical scavenging activity (RSA) and vitamin C levels of apple (*Malus domestica*) pomace and a local variety of jujube (*Ziziphus mauritiana*) pomace was evaluated after drying at 110 °C for 3 h in a hot air oven. The physico-chemical properties and functional properties of apple and jujube pomaces were also assessed. Prior to digestion, apple pomace displayed greater levels of TPC, RSA and vitamin C (17.30 ± 0.59 GAE/g DW, $81.16 \pm 3.27\%$, 0.078 ± 0.01 g/L, respectively) in comparison with jujube pomace (16.90 ± 0.66 GAE/g DW, $54.65 \pm 2.09\%$, 0.069 ± 0.01 g/L, respectively), whereas, TFC level was found to be higher in jujube pomace (19.22 ± 0.87 QE/g DW). After digestion, both samples showed an increase in TPC (56.17 ± 2.14 and 52.01 ± 2.18 GAE/g DW for apple and jujube pomaces) and TFC levels (48.45 ± 1.87 and 53.82 ± 2.34 QE/g DW for apple and jujube pomaces) and it was perceived almost 3 to 4 times higher than the TPC and TFC of the samples before digestion. But, RSA of the fruit pomaces were found to be affected by the in vitro digestion which was observed as 54.65 ± 2.09 and $81.16 \pm 3.27\%$ respectively for apple and jujube pomaces. It may be suggested that the fruit powders may be incorporated in developing new functional foods rich in bio

active compounds and thus can be utilized in different food applications.

Keywords In-vitro digestion · TPC · TFC · Fruit pomace · Bio availability

Introduction

Apple (*Malus domestica*) is a pomaceous fruit which comes under the family of *Rosaceae* and genus *Malus* which is rich in Malic acid. Apple is constituted mainly with water (84%) and fructose (14%) (USDA 2015), along with micro nutrients such as, dietary fiber, poly phenols and vitamins (O'Shea et al. 2015). It is one of the most consumed fruit worldwide and it has several health benefits when it is consumed regularly. From the studies, it was noted that the daily intake of apple reduces the possibility of coronary heart disease and stroke occurrence and potential benefits on vascular function and blood pressure (Bondonno et al. 2012).

Ziziphus mauritiana is an edible nut which has a long history and mainly known as jujube. It is also commonly called as Indian date and it comes under the family of *Rhamnaceae*. It is available in several forms including fresh, dried and processed. It is rich in micro nutrients such as vitamins, phosphorous, calcium, iron and poly phenols (Li et al. 2007). Jujube is used in conventional medical treatments and it was found to be effective against oxidative stress disorders such as cancers, coronary heart diseases and other degenerative disorders (Plastina et al. 2012).

Fruits and vegetables are considered as an important source of bioactive compounds and dietary fibers (Nayak et al. 2018a). Polyphenols which are showing potent

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antioxidant activity are grouped under phytochemicals and helpful in reducing the risk of several disorders such as cardio vascular disease, certain cancers and hypertension (Nayak et al. 2018b). Various studies conducted on the daily intake of fruits and vegetables have witnessed a beneficial effect in the decrement of certain chronic diseases. Even if significant levels of phenolic compounds are present in ingested food, the absorption rate is very less (0.5–1%) (Correa-Betanzo et al. 2014).

In recent years, the application of various phenolic compounds and flavonoids as natural antioxidants in different food products has been increased enormously. The health beneficial effects of phenolics were previously evaluated by various clinical tests which has several demerits, like time consuming, expensive and requiring real time monitoring throughout the process. In vitro models serve as an alternative to the clinical studies for analyzing the effectiveness of phenolic compounds, which provides excellent results within a short period of time. In vitro digestion technique can be used for promptly characterizing bio active compounds and it has the potential to replace the animal and human model as it is a rapid and economical method (Lee et al. 2016).

In vitro digestion technique is a stimulated process which is created outside the human body by providing the required conditions. It acts as an alternative for animals and human models which is often used for knowing the bioavailability of several metabolites in a food sample. Being an artificial process, it is bound to fail sometimes as in vivo process occurring inside the human body has many factors affecting it such as pH, temperature, food composition, structure as well as enzyme characteristics (Lee et al. 2016).

During the digestion process the food undergoes many changes and comes in contact with different enzymes which may change the components or structure of the food. Bioavailability and bioaccessibility of food ingredients are influenced by the physiochemical and biochemical reactions involved in the digestion process (Gunathilake et al. 2018). Mainly the bioaccessibility of polyphenols present in the fruits and vegetables are influenced by the different food processing technologies. Further, breakdown of food through mouth mastication, stomach digestion and intestinal digestion also affect the bioaccessibility of poly phenols when the food mixed with gastrointestinal secretions (Alminger et al. 2014).

Various research works were conducted on the in vitro gastro intestinal digestion of fruits and vegetables in the recent years, specifically on the bioactive compounds present in fruits and vegetables (Gunathilake et al. 2018).

The present work has been aimed (1). to estimate the physicochemical (pH, TA, TSS, color values, a_w and moisture content) and functional (Oil adsorption capacity,

Water holding capacity, and Swelling ability) properties of apple and jujube pomaces, (2). to compare the bio actives levels (TPC, TFC, RSA and vitamin C) of fruit pomaces before and after in vitro digestion.

Materials and methods

Plant materials

Fresh fruits (Apple and Jujube) were collected from a local market situated in Kokrajhar, Assam, India. Initially, fruits were cleaned using tap water and the inedible portions were removed. The fruit pomaces (apple pomace (AP) and jujube pomace (JP)) were obtained by grinding apple and jujube in a mixer grinder (Model: HL1632; Philips, Mumbai, India). Fruit pomaces were packed into airtight Ziploc bags and stored in freezer for 24 h at $-22\text{ }^\circ\text{C}$.

Chemicals

Folin & Ciocalteu's reagent (SRL), 2,2-diphenyl-1-picrylhydrazyl, pancreatin, (Sigma Aldrich, Bengaluru, Inida), α -amylase, Pepsin, (Himedia, Mumbai, Inida) K_2OH , KH_2O , CaCl_2 , HCl , NaHCO_3 , NaOH , (Merck, Mumbai, India), bile salt and other chemicals used in the research study were purchased from Sigma Aldrich (Bengaluru, India).

Fruit pomaces

Fruit pomace samples were thawed at room temperature overnight and were dried in a hot air oven (101E-LCD, ICON Instruments Company, New Delhi, India) at $110\text{ }^\circ\text{C}$ for 3 h (Gouw et al. 2017). The dried pomace was ground with a mixer grinder (Model: HL1632; Philips, Mumbai, India) and stored in air tight containers and kept in a desiccator till analysis.

Physiochemical analysis

Moisture content and water activity (a_w)

In the determination of moisture content, 10 g of sample (wet and dry pomace) was taken and dried in a hot air oven (101E-LCD, ICON Instruments Company, New Delhi, India) at the temperature of $105\text{ }^\circ\text{C}$. Samples were weighed after 3 h of drying and weighing was repeated after every 30 min until constant weights were achieved. The results were expressed on dry basis (AOAC 1995).

Water activity of fruit pomaces was determined using water activity Analyzer (Aqua lab Pre, Labcell Ltd, Hampshire, UK).

pH, titratable acidity (TA) and total soluble solid (TSS)

pH, TA, TSS of fruit pomaces were determined by following the modified method of Cavender et al. (2014). In this method, 5 g of pomace (wet or dried) was thoroughly mixed with 45 mL of distilled water. The mixture was filtered using Whatman #1 filter paper and the filtrate was used for the analysis. pH was determined by taking 20–30 mL of filtrate and using a digital pH meter (pH Testr 30, Eutech Instruments, Singapore). Titratable acidity (TA) of the filtrate was estimated by titrating it against 0.01 M NaOH (AOAC 2000). TA for apple and jujube pomaces was expressed in terms of malic acid and citric acid, respectively. Total soluble substances (TSS) was determined using a handheld refractometer (MASTER-500, Atago, Japan) and it was presented in terms of °Brix (Nayak et al. 2018a).

Color measurement

The colour of Fruit Pomaces were determined by Hunter colorimeter (D25 LT, Hunterlab, USA) and color values of the samples were represented by L*, a* and b* values (Michalska et al. 2016).

Functional properties of dried fruit pomaces

Oil adsorption Capacity (OAC)

Fruit pomaces were evaluated for their oil absorption capacity by using the modified method of Femania et al. 1997. In this method, 1 g of FP was added to 10 mL of olive oil in a centrifuge tube and incubated overnight at room temperature. Samples were centrifuged (REMI C-24BL, Mumbai, India) at 1500 rpm for 5 min. After centrifugation, the supernatant was discarded and the adsorbed oil present in the samples were calculated. The results of the experiment were expressed in terms of g oil/g dry weight of sample.

Water-holding capacity (WHC)

The estimation of WHC of fruit pomaces were carried out based on the method of Sudha et al. (2007) with minor changes. In this analysis, 30 mL of distilled water was added to 1 g of dried FP and centrifuged at 5000 rpm for 30 min. The excess water was drained. The quantity of absorbed water was estimated by reweighing the samples and the results were given as g water/g dry weight of sample (Reißner et al. 2019).

Swelling ability (SA)

Estimation of swelling ability of FP samples was accomplished by following the altered method of Femenia et al. (1997). During the analysis, 1 g of FP sample was added to 20 mL of distilled water and the solution was vigorously. The mixture was kept in room temperature for establishing the swelling of fibers in the FP samples. The samples were weighed after discarding the additional water present in the sample and SA was indicated as ml/g of dry weight of sample.

Extraction of phenolics from dried fruit pomace

Initially, 3 g of dried FP sample was taken along with 30 mL of 60% methanol acidified with 1% glacial acetic acid and the resulting mixture was placed in an ultra sonicator (VGT-1730QTD, Guangdong GT Ultrasonic Co., Ltd., China) for 20 min. After ultra sonication, the mixture was filtered using Whatman #1 filter paper. Then, the volatile compounds present in the filtrate was removed by keeping it in a Rotary vacuum evaporator (ROTA VAP R-210, BUTCHI, Rose Scientific Ltd., Canada) at 50 °C for 10 min. 25 mL of distilled water was added to the sample to carry out the further analysis (Gouw et al. 2017).

Total phenolic content (TPC)

Folin-Ciocalteu method (Nayak et al. 2018b) was followed to assess the TPC levels of FP. Equal portions of sample and reagent (5 mL) were mixed along with 7.5 mL of distilled water. 3 mL of 20% sodium carbonate solution was added to the mixture and vortexed well. Sample was kept in a water bath for 20 min at 40 °C. After incubation, sample was cooled and the absorbance of the solution was taken at 784 nm by using a UV/VIS spectrophotometer (Lambda 35, PerkinElmer, United States). Total phenol content was expressed as mg gallic acid equivalents (GAE)/g DW.

Total flavonoids content (TFC)

Total flavonoid levels were calculated by applying the method of Nayak et al. (2017). 0.25 mL of sample was mixed with 1.25 mL of distilled water and 0.075 mL of NaNO₂ (5%) solution. After 5 min, 0.15 mL of AlCl₃ (10%) was added to the mixture and 0.5 mL of 1 M NaOH was mixed with the solution after 6 min. The resulting mixture was incubated at room temperature for 30 min and the absorbance was measured at 510 nm using a UV/VIS spectrophotometer. The results were produced as mg quercetin equivalents (GAE)/g DW.

Radical scavenging activity (RSA)

RSA of fruit pomace extracts was estimated by using DPPH assay (Radha krishnan et al. 2013). During the estimation, 0.5 mL of sample was mixed with 4 mL of 0.004% methanolic DPPH solution. Sample was kept in dark conditions at room temperature for 30 min. Then the absorbance of sample was noted at 517 nm using UV/VIS Spectrophotometer (Lambda 35, PerkinElmer, United States) and RSA was reported as percentage of DPPH radicals quenched.

Vitamin C level in samples was determined according to the method of Nayak et al. (2018b).

In-vitro gastrointestinal digestion

Stimulated gastrointestinal digestion of fruit pomaces was executed by applying the method of Eriksen et al. (2017) with suitable changes. It comprises three phases, namely mouth mastication, stomach and intestinal digestion.

In the first phase, FP samples (1 g) were taken along with, 8.5 mL 0.05 M phosphate buffer (pH- 7), 0.5 mL of α -amylase (20 FAU/g), and 150 μ L of 50 mM CaCl_2 . The contents were mixed well by keeping in a shaker water bath (LabTech, Daihan Lab Eriksen Eriksen ech, India), which was maintained at 37 °C and 50 rpm for 2 min.

The samples were removed after 2 min to proceed for stomach digestion. During this experiment, the samples were added with 5 mL of distilled water, 1 mL of 0.2% porcine pepsin solution, and 30 μ L of 50 mM CaCl_2 . The pH of mixtures was attuned to 3 with the addition of 1 M HCl. The final volume of the solutions has been made up to 20 mL by adding distilled water and the contents of the solutions were mixed well in the water bath at 200 rpm and 37 °C for 2 h.

After incubation, the samples were taken out and added with 10 mL of 0.05 M phosphate buffer (pH 7.0), 3.0 mL of duodenal juice (12.5 g of bile salts and 2 g of pancreatin in 60 mL 0.1 M of NaHCO_3) and 240 μ L of 50 mM CaCl_2 . The pH of the mixtures was attuned to 7 by adding 1 M NaOH. The final volume has been made up to 40 ml by using 0.05 M phosphate buffer (pH 7.0). The solutions were kept in a shaking water bath at 200 rpm and 37 °C for 2 h. After incubation, the sample were centrifuged at 6000 rpm for 30 min at 4 °C by using a coolin centrifuge (REMI C-24BL, Mumbai, India). The supernatant was separated to calculate TPC, TFC, RSA and vitamin C levels.

Statistical analysis

All analysis in this study was performed in triplicates. The experimental results were given as mean value \pm standard

deviation. The analysis of experimental results was accomplished with SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) by ANOVA at $p < 0.05$, LSD was used to compute the variations in between mean values.

Result and discussion

Physicochemical analysis

The physicochemical properties were depended on the species of fruits and the region where it is being produced. The functional properties were based on fiber traits, comprising crystalline structure, surface aspects and hydrophobic behavior and it might also differ on the type of fruits or vegetables investigated (Femenia et al. 1997).

The experimental data for the physicochemical properties of fruit pomaces were given in Table 1. Moisture content of wet apple pomace (WAP), wet jujube pomace (WJP) were recorded as 84.41 ± 3.54 and $81.34 \pm 2.78\%$ respectively and the levels were varied significantly ($p < 0.05$). The major component of both pomaces is pulp which largely comprises of vital levels of dietary fibers which possess large quantities of water. The differences in moisture levels also due to the enzymatic deterioration of pectin which changes the distribution of cellular compounds in pomaces.

The water activity (a_w) values of wet FP were recorded as 0.98 ± 0.03 and 0.99 ± 0.02 for WAP and WJP respectively as given in Table 1 and the values were found to be insignificant ($p < 0.05$). The a_w values of FP indicated that they are very much prone to chemical and biological deteriorative reactions which leads to the spoilage of FPs (Gouw et al. 2017). Therefore, for a longer storage FP, a_w has to be reduced by suitable processing techniques (drying).

Titrateable acidity of wet fruit pomace was displayed in Table 1 and it can be seen that TA level of apple pomace ($0.44 \pm 0.02\%$) was significantly higher than ($p < 0.05$) jujube pomace. The variations in TA may be due to the higher content of malic acid in apple pomace as well as the differences in transfer of organic acids from fruit juices to fruit pomaces. Total soluble solids (TSS) levels of wet FPs were estimated as $12.00 \pm 0.10\%$ and $7.00 \pm 0.10\%$ and varied significantly ($p < 0.05$). From the TSS levels it can be understood that the apple pomace has high sugar content and other soluble components than jujube pomace.

The moisture content of dried fruit pomaces displayed similar trend as wet fruit pomaces, where dried apple pomace showed higher moisture content ($15.78 \pm 0.62\%$) than dried jujube pomace ($14.85 \pm 0.55\%$) as given in Table 1. The a_w values of dried FPs were calculated as 0.34 ± 0.02 and 0.31 ± 0.01 for apple and jujube pomace,

Table 1 Physiochemical properties of Apple and Jujube pomaces (wet and dry)

Sample/parameters	Apple pomace(AP)		Jujube pomace(JP)	
	WAP	DAP	WJP	DJP
Moisture content (%)	84.41 ± 3.54 ^a	15.78 ± 0.62 ^b	81.34 ± 2.78 ^{ab}	14.85 ± 0.55 ^c
Water activity(a _w)	0.98 ± 0.03 ^a	0.34 ± 0.02 ^b	0.99 ± 0.02 ^a	0.31 ± 0.01 ^b
pH	4.72 ± 0.14 ^a	4.25 ± 0.12 ^b	4.54 ± 0.09 ^a	4.13 ± 0.13 ^b
Titrateable acidity (%)	0.44 ± 0.02 ^a	0.11 ± 0.01 ^b	0.30 ± 0.03 ^c	0.10 ± 0.01 ^b
TSS(°brix)	12.00 ± 0.10 ^a	7.00 ± 0.00 ^b	2.00 ± 0.10 ^c	4.00 ± 0.10 ^d
Color values				
L*	38.25 ± 1.09 ^a	35.41 ± 1.13 ^b	42.14 ± 1.29 ^c	33.31 ± 1.32 ^b
a*	11.18 ± 0.31 ^a	10.01 ± 0.28 ^a	9.46 ± 0.24 ^a	3.91 ± 0.08 ^b
b*	32.86 ± 0.92 ^a	32.09 ± 1.24 ^a	26.86 ± 0.81 ^b	23.62 ± 0.79 ^c

Values with different letters in the same row (a–c) are significantly different ($p < 0.05$) from each other
WAP wet apple pomace; DAP dried apple pomace; WJP wet jujube pomace; DJP dried jujube pomace

respectively. From the a_w values, it can be seen that the dried FPs were stable for longer storage periods as the deteriorative reactions may not occur (Gouw et al. 2017). TSS values of for dry apple and jujube pomace was obtained as $7.00 \pm 0.00\%$ and $4.00 \pm 0.10\%$, respectively and showed significant differences ($p < 0.05$) similar to wet pomaces. Similarly TA of dried apple pomace ($0.11 \pm 0.01\%$) was insignificantly higher than dried jujube pomace ($0.10 \pm 0.01\%$).

The acceptance of the food product has been decided by the color and it plays a major role in deciding the quality of the dried food products. The color values of wet and dry pomaces (apple and jujube) was presented in the Table 1. As observed from the table, the color values (L^* , a^* , b^*) of wet and dry apple pomaces (WAP & DAP) did not show any significant variations except L^* values. After drying, the L^* value of was found to be reduced and obtained as 38.25 ± 1.09 to 35.41 ± 1.13 for WAP and DAP. The reduction in L^* value indicated the darkening of the dried apple pomaces. The decrease in L^* values (lightness) may be due to the oxidative reaction that occur during the drying process which was mediated by the drying air temperature (Doymaz 2017) and minor deviations in the color of WAP and DAP might be due to the browning reactions that occur during drying process (Kayran and Doymaz 2017). The color values (L^* , a^* , b^*) of wet and dry jujube pomaces (WJP & DJP) were found to be differed significantly. Before drying, L^* , a^* and b^* values of jujube pomace (WJP) was recorded as 42.14 ± 1.29 , 9.46 ± 0.24 and 26.86 ± 0.81 , respectively. The values decreased significantly after drying and observed as 33.31 ± 1.32 , 3.91 ± 0.08 and 23.62 ± 0.79 for wet jujube pomace. Compounds like chlorophyll, lycopene and carotene, which are present peels and flesh of fresh fruits are responsible for color. The decrease in a^* values may be due to the occurrence of browning reactions during

the drying process and the degradation of reddish pigments. The reduction in b^* value (yellowness) of the fruit pomace might be attribute to the drying air temperature, degradation of carotenoid pigments and also due to the non-enzymatic browning reactions and the development of browning compounds (Doymaz 2017).

Functional properties of apple and jujube pomaces

The experimental results for the functional properties of fruit pomaces has been represented in Table 2. Oil absorption capacity was the quantification of oil retention in foods and rely upon particle size rather than chemical constituents or molecular affinity to oil (Yalegama et al. 2013). Among the pomace varieties, oil absorption capacity differs significantly ($p < 0.05$) and recorded as 2.08 ± 0.08 and 2.49 ± 0.09 g oil/g DW for dried apple and jujube pomaces, respectively. The differences in the oil absorption capacities might be attributed to the lignin content (insoluble dietary fiber) which might play some role in oil absorption of fruit pomaces (Rana et al. 2015).

The water holding capacities (WHC) of pomaces showed significant ($p < 0.05$) variations and among them, apple pomace exhibited higher WHC (4.40 ± 0.14 g

Table 2 Functional properties of Apple and Jujube pomaces

Sample/parameters	DAP	DJP
OAC(g oil/g dry weight (DW))	2.08 ± 0.08 ^a	2.49 ± 0.09 ^b
WHC(g water/g DW)	4.40 ± 0.14 ^a	2.61 ± 0.07 ^b
SA(mL/g DW)	13.8 ± 0.48 ^a	11.99 ± 0.39 ^b

Values with different letters in the same row (a–c) are significantly different ($p < 0.05$) from each other

OAC oil absorption capacity; WHC water holding capacity; SA swelling ability; DAP dried apple pomace; DJP dried jujube pomace

water/g DW) as given in Table 2. The lower WHC in jujube pomace might be due to the greater degradation of pectin as reported by Raghavendra et al. (2004). In addition, fibers which comprise large quantities of primary cell wall compounds (like pectin) may exhibit high WHC in comparison to fibers which contain more amounts secondary wall compounds (like lignin).

The swelling ability (SA) of pomaces differed significantly ($p < 0.05$) and apple pomace registered higher SA (13.8 ± 0.48 mL/g DW) in comparison to jujube pomace (Table 2). As reported earlier (Gouw et al. 2017), the higher SA of apple pomace was due to the presence of large fibers and voids which offered large area for water binding. In contrast, jujube pomace was consisted largely with seeds and constituted primarily with cellulose and lignin, thus poor interaction with water (Gouw et al. 2017). The above results showed that dried apple pomace has more dietary fibers when compared to dried jujube pomace. This can be verified as the jujube mainly consists of a large seed and is less fibrous than apple.

Effect of in vitro digestion (IVD) on TPC, TFC, RSA and vitamin C levels

Fruits and fruit products were studied to utilize them as food sources in various research reports. The bioavailability of plant products was not discussed in most of these studies. But, it is important to know the bioavailability of bio active compounds present in fruits. The bioavailability of phenolic substances and antioxidants was based on their release from food matrix during the digestion process. So, it was important to know the availability of these bio active compounds after digestion and several studies indicated that bioavailability of some antioxidants is very low owing to their solubility and metabolism in the gastro intestinal tract. Therefore, the changes in the contents of TPC, TFC and RSA has to be studied in order to understand their bioavailability during digestion (He et al. 2017; Kamiloglu et al. 2014).

The data for TPC, TFC, RSA and vitamin C levels in fruit pomaces (before and after in-vitro digestion) were presented in Fig. 1a–d. Before in vitro digestion (IVD), total phenolic content in dried apple pomace and dried jujube pomace were observed as 17.30 ± 0.59 and 16.90 ± 0.66 GAE/g DW respectively. TPC of FPs was influenced by type of extraction process, type of solvent used and phenolic levels in fruits (Turkmen et al. 2006; Wang et al. 2016). Loss of phenols may also occur during extraction process which may not be in significant quantities. After IVD process, a significant ($p < 0.05$) increase in TPC was observed (Fig. 1a) and the levels were found to be 56.17 ± 2.14 and 52.01 ± 2.18 GAE/g DW for apple and jujube pomace, respectively. From the results, it can be

seen that IVD enhanced the release of different phenolic substances from the fruit pomace. Similarly, Chen et al. (2016) reported that gastric conditions might improve the extractability of phenolic compounds from fruit pomaces. The experimental data from our research work was similar to the work carried out by Gouw et al. (2017).

When fruits are consumed orally, they are exposed to gastrointestinal (GI) conditions. The bioavailability of various compounds may be altered due to the pH variations, action of different enzymes, body temperature and other physical and biochemical conditions. It is also essential that the bio active compounds to be ingested into the systemic circulation and attain the desired tissues at adequate levels (Barak et al. 2019). In the present research study, the levels of bio active compounds were increased after IVD. From the results, it can be inferred that the phenolic compounds in the fruit pomaces have high stability in the GI tract. It might be correlated with the continuous liberation of phenolic compounds from protein other macromolecules during the digestion process which changes the chemical structure as well as functional properties. It was also assumed that the increase in the polyphenol levels could be due to the hydrolysis reaction s which occurs during the digestion process (Durak et al. 2014).

Before digestion, level of total flavonoids (TFC) in dried apple and jujube pomace was varied significantly ($p < 0.05$) and found as 13.32 ± 0.34 and 19.22 ± 0.87 QE/g DW (Fig. 1b). From the figure, it can be observed that the TFC levels were increased significantly ($p < 0.05$) after IVD, a trend similar to TPC. The significant rise in TFC levels after IVD, might be attributed to the fact that the flavonoid compounds which were bound to protein or fiber were discharged as a result of digestive enzymes (Zhou et al. 2016). Additionally, the changes in the polyphenols contents in each section of IVD could also be a factor in the TFC variations (Gutiérrez-Grijalva et al. 2019).

Radical scavenging activities of FPs were recorded as 91.68 ± 3.78 and $84.71 \pm 3.5\%$ for apple and jujube pomaces, respectively, before IVD. The values were reduced to 54.65 ± 2.09 and $81.16 \pm 3.27\%$ respectively after digestion. The results of our work was found to similar with the research study of Lucas-González et al. (2018), where it was reported that a decrease in the antioxidant activities of persimmon fruit co-products, might be a result of chemicals reactions like oxidation, polymerization or due to interactions between different dietary compounds like fiber, protein and carbohydrates.

The decrease in RSA might also be a result of various kinds of antiradical compounds that are present in the fruit pomace. The mechanisms and kinetic reactions of this antiradical compound may affect the composition in the

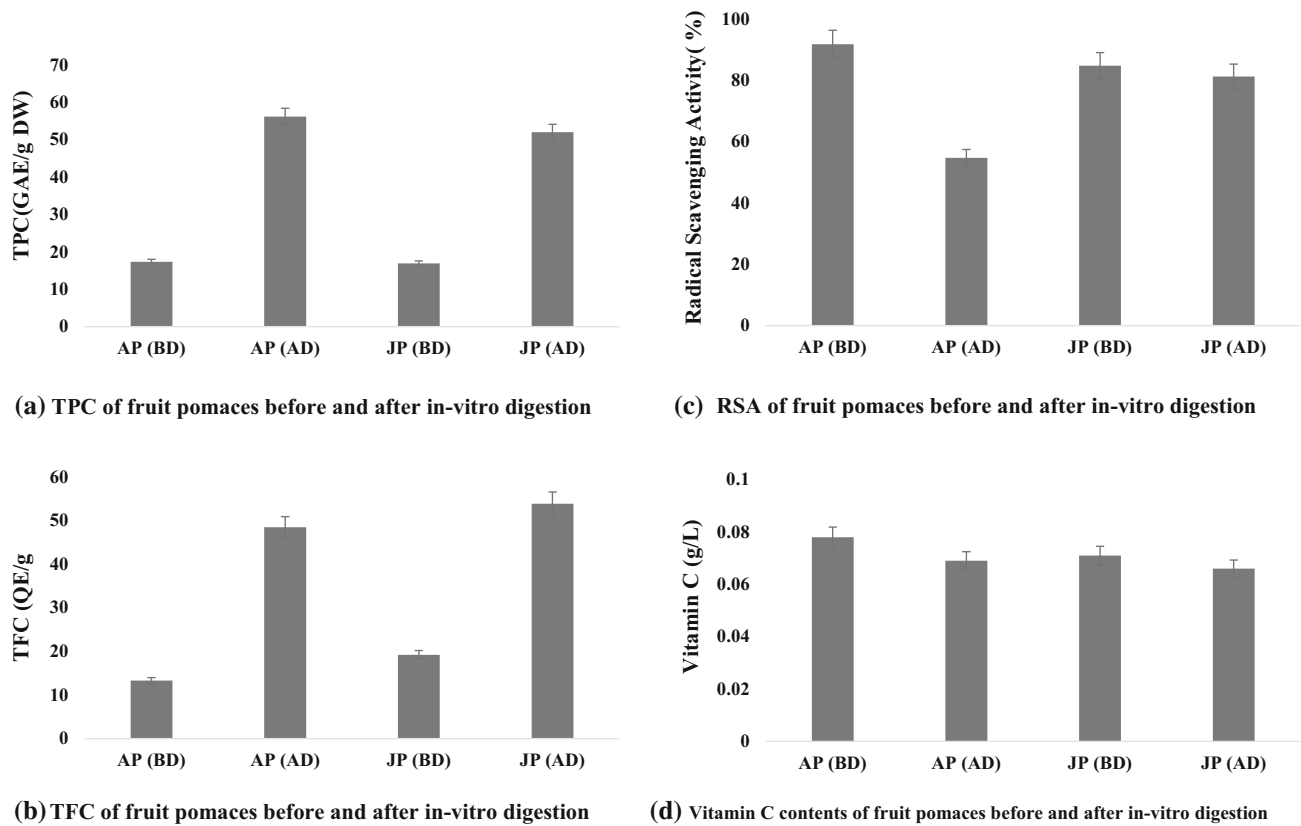


Fig. 1 **a** TPC of fruit pomaces before and after in-vitro digestion, **b** TFC of fruit pomaces before and after in-vitro digestion, **c** RSA of fruit pomaces before and after in-vitro digestion, **d** Vitamin C contents of fruit pomaces before and after in-vitro digestion. *AP (BD)*

apple pomace (before digestion); *AP (AD)* apple pomace (after digestion); *JP (BD)* jujube pomace (before digestion); *JP (AD)* jujube pomace (after digestion)

fruit pomaces (Brand-Williams et al. 1995). Research study by Bermúdez-Soto et al. (2007) showed that antioxidants are highly sensitive to pH and can be easily degraded which results an overall loss in antioxidant capacity after in vitro digestion. The results are not in accordance to the study Gouw et al. (2017) who reported an increase in the RSA of DAP where as in our study there is a large fall in the RSA which might be the result of the species of samples as well as the procedure. It was well known that the racemization of constituents has depend on pH (Wootton-Beard and Ryan 2011). So, it might change their biological activities and may reduce the antioxidant properties of the bioactive compounds during initial phase of the digestion process at acidic pH (stomach digestion). Further, biomolecules may be less active at pH 7.4 in the intestinal digestion as racemization may rise with pH (Jamali et al. 2008; Wootton-Beard and Ryan 2011). Therefore, the antioxidant activities of bioactive compounds were decreased after the completion of IVD.

The level of vitamin C was found to be higher in apple pomace (0.078 ± 0.002 g/L vitamin) in comparison with jujube pomace (0.069 ± 0.01 g/L vitamin) and no significant changes in the vitamin C content after digestion was

observed i.e. 0.071 ± 0.01 and 0.066 ± 0.002 g/L for apple and jujube pomaces, respectively. The results indicated that vitamin C levels was not much affected by the enzymes or the untangled fiber. Vitamin C being one of the antioxidant was also affected by the in vitro digestion and has same effect like that in antioxidant activity.

Conclusion

From this research work, it was understood that the levels of different constituents in fruit pomaces has been varied depend on the location where the fruits were produced. The physicochemical and functional properties of fruit pomace depend upon the preparation, the micro structure as well as the amount of fiber present in it. The total phenol content of fruit pomace depends upon the efficiency of extraction and is also related to fiber content of the fruit. The phenolics in fruit are present in the bound form. These bound phenolics can be liberated by stimulated gastrointestinal digestion. The study also differentiated between the physicochemical and functional properties. The study successfully demonstrated the changes in the dried fruit pomaces after it

undergoes gastrointestinal digestion and highlighted the effectiveness of *in vitro* digestion. The results also exhibited IVD plays a vital role in increasing the total phenol as well as total flavonoid contents. The results might be helpful in development of new functional food products as the fruit pomaces contains good amounts of anti-oxidants which were involved in the prevention of several diseases. This research work delivered some useful data regarding the polyphenols as well as antioxidant activities of apple and jujube pomaces before and after *in vitro* digestion with their physicochemical and functional properties which can be beneficial for consumers, nutritionists, and in food market.

Compliance with ethical standards

Conflicts of interest All the authors declare no conflict of interests.

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