Comparison of storability and seasonal changes on new flavonoids, polyphenolic acids and terpenoid compounds of *Citrus paradisi* (grapefruit) cv. shamber through advance methods

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Abstract

The significance of grapefruit is laying in its unique medicinal values and health related compounds. This article discusses the influence of seasonal variations and storage periods on the synthesis and accumulation of new polyphenolic compounds, terpenes, flavonoids and sugars profiling of grapefruit (*Citrus paradisi*) cv. shamber juice were evaluated under advance techniques. The individual sugar profiling of (total sugar, fructose, glucose and sucrose) individual phenolic acids and essential groups of terpene compounds measured at five harvesting times, from early to late stages and compare to storage days of (0, 15, 30, 35, 45 and 65). The higher contents of flavonoids were obtained in December harvested fruits while in comparison the 45 and 65 days the contents were reduced however in others days the contents were maintained the higher contents of Limonene, Quercetin, Perillyl alcohol and Monoterpenes were measured in December harvested fruits the 35 day of storage periods showed the constant contents of terpenes and little reduction of terpenoid at 45 and 65 days of storage. It may conclude that the mid date was best for good health fruits while the all of these compounds were present in higher amount at 35 day of storage.

Keywords Antioxidants · Carotenoids · Flavonoids · Harvest times · Polyphenols

Introduction

Citrus fruits, being a perennial and tropical crop, subjected to significant seasonal variations of the climate changes during its growth and developmental stages were noted [1]. Early and late harvesting dates showed reducing of essential compounds [1, 2]. Citrus fruits cultivated in more than 64

countries of the world, including in various areas of Pakistan with a total production of 105.4 million tons annually. Grapefruit (*Citrus paradisi* Macf.) is a natural cross between pummelo as a seed parent and sweet orange or some other similar parent's pollen donor are commonly used [3]. Bioactive compounds are major class of secondary metabolites with contained 9000 structures, but accessible and famous in grapefruit juice and its parts are famous all over the world [2]. The unique character in grapefruit is its flavonoids and phenolic compounds derived from 2-phenylchromane commonly a massive amount of polyphenolic compounds were found in juices, peel, rags and seeds [1]. The environmental conditions could be more or less limiting factors for its bioactive compounds synthesis, accumulation, and formation, rely on the geographical area, growing season and harvesting times [4]. Grapefruit has a unique value of essential nutrients which are protective against several chronic diseases of the human body [5]. Seasonal variation is a critical factor for its quality changes during the growth and developmental process due to constantly the cell maturation process [4]. The grape fruits harvested in early august, the harvested

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fruit direct influence the quality and storage ability [2]. The storage and quality reducing has a direct linked with these harvesting dates if proper harvested fruits then commercial storage periods maintained the quality and storage potential [4]. Pakistan and worldwide issue of early harvested fruit to harvest their fruits early to get high prices in local or exporting markets [3]. The early harvesting causes some fruit quality problems, including undersized fruits and low internal quality (e.g., high acidity, low total soluble solids (TSS), low juice content, etc.), lower sugar profiling, unstable bioactive compounds [6]. Fruit marketable quality is largely determined by the stage of development of fruit at harvest time consequently, fruit picked while immature or likely to be small, poorly colored, off-flavored and more susceptible to diseases when stored and less average life of fruit have been noted in mostly grapefruit during storage process, over-mature fruit, besides having a shorten storage life, with poor storage quality is susceptible to granulation, chilling injury and fruit rotting during commercial storage process. The storage periods are major concern of quality and shelf life early and late harvested fruits showed lower values of health related compounds [6]. The maturity had a relationship with these bioactive compounds and immature and early harvested fruits showed lower concentrations of these essential compounds after harvesting [7].

A detailed literature search reveals that little/ none of investigations have been reported about the impact of harvesting times and storage changes on grapefruit juice related to active phytochemicals and terpene compounds. The aim of this study to evaluate the seasonal variations and storage stability on various sugar profiling, flavonoids, and terpene compounds. The grapefruit is top fruit in world for its unique health related compounds the study is faced to minimize the fatalities related to seasonal variations and storage changes on these essential compounds.

Materials and methods

Plants sampling and experimental site

The sampling of fruits were collected as reported earlier by Ahmed et al. [2] with different harvesting dates viz. 1st September, 1st October, 1st November, 1st December and 1st January. Fruits were randomly harvested from selected trees with fruit clipper and brought to the Pomology Lab., Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. All chemicals analytical grades were purchased from Merck. Germany. Deionized water received from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Fruit harvesting and storage

Fruits were harvested on the month of January and Stored at 6 °C for 65 days, the fruits were compare the others days of storages.

Washing and cleaning of grape fruits

Harvest fruit were washed using sodium hypochloric solution 100 ppm in a plastic tube to remove adhering extraneous matters on the fruit surface and sorting was carried out unsound/damaged fruits and then uniform colored grape fruits were separately.

Fungicide applications

Thiabendazole (TBZ) @1000 ppm was applied in the room temperature at 30 °C for 10 min for controlling of diseases in storage periods.

Storage temperature and days of storage

These fruits were placed at temperatures i.e. 6 °C at 85–90% RH in cold chambers equipped with automatic relative humidity and temperature control system. The grape fruit samples were randomly drawn after every 0, 15, 30, 35, 45 and 65 days interval during the whole periods of storage.

Profiling of individual sugar in grapefruit juice

The sugar profiling of (starch, fructose, glucose and sucrose) investigated by the HPLC method as early reported method of AOAC [8]. The sugars were extracted into 60% methanol and passed through C-18 column and then filtrated of samples were in a 0.46 mm of disc nylon, the quantitation and separation were carried out on a bonded amino column with a mobile phase of H₂O and CH₃CN (20/80 v:v). The evaporative light scattering detector (ELSD) detector, Sedex 60 lt ELSD (Sedere) and an interface LCNET II/ADC (JASCO, Japan) was used. The separation of sugars were performed using an amino column Kromasil 100 5NH₂ (250 mm × 4.6 mm) (Akzo Nobel, Bohus, Sweden) with a mobile phase of 77% acetonitrile and 23% HPLC grade water, degassed and ultra-sonicated was added. The analysis conditions were held constant at a flow rate of 1.8 mL with time of 1 min at 20 °C under a pressure of 13.2 kPa. The injection volume was 20 µL.

Preparation and running of sample in HPLC-DAD

The individual phenolic acids and flavonoids analyzed through high-performance liquid chromatography (HPLC). The reserved phase was carried out in an isocratic condition using a column of C18. The mobile phase of acetic acid and methanol at a ratio of (70:40 v/v) were used. The injected volume was 6 µl and the range were measured at 290 nm. The flow rate was 2.0 during the running time of bioactive compounds. The peaks and concentrations calculated by comparing the peak areas with reference compounds which were run under the same elution conditions. The data recorded in the winch room.

HPLC-DAD method for induvial phenolic acids and flavonoids in grapefruit juice

The induvial phenolic acid and flavonoids were determined as reported method of Ahmed et al. [1] with some slightly modifications were done the HP 1100 series from Agilent Technologies, including a degasser, a binary pump delivery system, and an automatic liquid sampler, was used and coupled with diode array (DAD). The 5 µl of filtered sample of grapefruit juice was injected in a reverse phase column of Poroshell 120 SB-C18 (3 × 100 mm, 2.7 µm) from Phenomenex, and the separation was carried out using as a mobile phase A acidified water (1% acetic acid) and as mobile phase B acetonitrile. The following multistep linear gradient was applied: 0 min, 5% B; 12.5 min, 30% B; 17.5 min, 60% B; 22 min, 5% B. The initial conditions were maintained for 5 min. The flow rate was set at 0.6 ml, min⁻¹ throughout the gradient. UV spectra were recorded from 210 to 600 nm, while the chromatograms were registered at 280 and 330 nm. Separation was carried out at 25 °C. The extracted compounds were identified by analyzing MS spectra and quantified by UV chromatograms. The compounds were properly verified with standard MS detectors.

Statistical analysis

The results were submitted to a factorial analysis of variance and the means values were compared by using separated duncan's multiple range test was apply in this study. The number of replicates for each analyzed sample was three [9], the physical and maturity were calculated by non-linear regression, based upon their concentration-response curve of each sample by using graph Pad Prism 5 was used. The data of flavonoids and phenolic acids were further verified by the version of 1.7 (Sciex City Foster USA) software was used. The control data acquisition was evaluated with peaks of samples with further measured and check by the (Multi-Quant 2.1.1.2 Foster CA, USA) software used. The peak areas, standards curves, descriptive statistics calculations were performed using advanced software of Win-NonL in ® version 6 was apply [9].

Results

Comparison of harvesting dates and storage periods on individual sugar profiling of grapefruit (Shamber) juice

The different sugars contents were found in *Citrus × paradisi* cv Shamber analyzed under several harvesting times leading to early mature to full maturity stage and storage periods (0, 15, 30, 45 and 65) days whereas results are reported in Table 1. Results showed that maximum total sugars (7.14 g), Fructose (1.5 g), Glucose (1.6 g), and Sucrose (3.3 g) were observed in the fruits, harvested in month of December in comparison of other harvesting dates like September, October, November and January harvested fruits. The fruits were harvested in month of January and storage for period of 65 days while the storage periods showed the contents were increased gradually till the storage period of 65 days with the values of Fructose (1.8 g), Glucose (1.9 g), and Sucrose (3.6 g) were noted at the end of storage period.

Table 1 Comparison of harvesting dates and cold storage changes of sugar profiling in grapefruit (*Citrus × paradisi*) cv. Shamber

Sugars (g)	Harvesting dates					Storage periods					
	Sep. 1st	Oct. 1st	Nov. 1st	Dec. 1st	Jan. 1st	0 DAS	15 DAS	30 DAS	35 DAS	45 DAS	65 DAS
Total sugar	4.4 e	5.0 d	5.1 c	7.14 a	6.3.0 b	6.3.0 f	6.40 e	6.5.0 d	6.6.0 c	6.7.0 b	7.0.0 a
Fructose	0.9 e	0.10 d	1.1 c	1.5 a	1.3 b	1.3 f	1.4 e	1.5 d	1.6 c	1.7 b	1.8 a
Glucose	0.11 e	0.14 d	1.0 c	1.6 a	1.3 b	1.3 f	1.5 e	1.6 d	1.7 c	1.8 b	1.9 a
Sucrose	2.6 e	2.8 d	3.0 c	3.3 a	3.1 b	3.1 f	3.2 e	3.3 d	3.4 c	3.5 b	3.6 a

The results are presented as mean based on $n=3$, *LSD* Least significant differences at ($p<0.05$). Different letters within column indicate significant differences between harvesting times and storage periods ($p<0.05$)

Effects of different harvesting dates and storage periods changes on individuals polyphenol acids in grapefruit (*Citrus × paradisi*) cv. shamber

The significant changes were recorded in polyphenolic acids with respect to the harvesting times and storage periods for shamber grapefruit juice which were maximum in the month of December while lowers Hydroxycinnamic acids (0.11 mg/100 g) was recorded in month of September harvested periods. The Ferulic acid were least but statistically significant results (1.5 mg/100 g) in month of December followed by 1.4 mg/100 g during January shown in (Table 2). The similar trends were found for Protocatechuic acid and Neochlorogenic acid from same harvested fruits in months of December. The storage periods of 15, 30 and 35 days showed a similar values however the storage periods of 45 and 65 days of stored fruits showed little reduction of all polyphenol acids while the Hydroxycinnamic acids showed the value of 1.0 at the 65 day of stored fruits.

HPLC-DAD analysis of seasonal variation and storage periods on structural terpene compounds in shamber grapefruit juice

The storage periods and harvesting dates were compare on structural terpene changes through HPLC analysis. The Limonene, Quercetin, Perillyl alcohol and Monoterpenes were same value on 15, 30 and 35 day of storage periods.

The 45 and 65 day of storage showed the reduction of compounds. The comparison of early and late harvesting date's showed reduction of terpene compounds so the fruit harvested at January the constant values were noted in these storage periods while the lower values of all compounds at 65 day of storage shown in (Table 3). The chromatogram of terepene compounds were shown in Fig. 1.

Effects of Individual's flavonoids (flavones, flavonols & flavanols or catechins) isolations process through HPLC-DAD methods

The 12 individual's flavonoids were isolated in grapefruit juice through HPLC-DAD methods, the storage periods and variations of harvesting times were compare in this study the flavonoids, flavones flavonols, and catechins shown in (Table 4). The level of Hesperidin varied significantly, increased from 2 mg/100 g FW to 6 mg/100 g FW from early to late in the season. The January harvested fruits were stored and compare the hesperidin level the constant level of 5 mg/100 g FW till the stored period of 35 day while the days of 45 and 65 showed reduction of hesperidin (4 to 3.5 mg/100 g FW). The contents of Narirutin and Neohesperidin were ranged from 40 mg/kg to 90 mg/kg and 6 to 14 mg/kg respectively. Similar the reduction of these two compounds in 45 and 65 day of storage periods. Neoponcirin and Rutin in all harvested fruits did not show any contents in harvested fruit (Table 4). Nobiletin was higher in December

Table 2 Means of phenolic acids in grapefruit (*Citrus × paradisi*) cv. Shamber at various harvested dates and storage periods (mg/100 g)

Separate Phenolic compounds (mg/100 g)	Harvesting dates					Storage periods					
	Sep. 1st	Oct. 1st	Nov. 1st	Dec. 1st	Jan. 1st	0 DAS	15 DAS	30 DAS	35 DAS	45 DAS	65DAS
Hydroxycinnamic acids	0.11 d	1.1 c	1.2 b	2.3 a	1.2 b	1.2 a	1.2 a	1.2 a	1.2a	1.1 b	1.0 c
Ferulic, acids	1.1 e	1.2 d	1.4 c	3.5 a	1.4 b	1.4 a	1.4 a	1.4 a	1.4 a	1.3 b	1.2 c
Protocatechuic acid	1.3 d	1.4 c	1.15 c	2.6 a	1.5 b	1.5 b	1.5 a	1.5 a	1.5 a	1.3 b	1.2 c
Neochlorogenic acid	1.2 e	1.3 d	1.4 c	2.6 a	1.5 b	1.5 a	1.5 a	1.5 a	1.5 a	1.3 b	1.2 c

The results are presented as mean based on $n=3$, *LSD* Least significant differences at ($p<0.05$). Different letters within column indicate significant differences between harvesting times and storage periods ($p<0.05$)

Table 3 Means of terepene compounds in grapefruit (*Citrus × paradisi*) cv. Shamber at various harvested dates and Storage periods mg/100 g

Terepene compounds	Harvesting dates					Storage periods					
	Sep. 1st	Oct. 1st	Nov. 1st	Dec. 1st	Jan. 1st	0 DAS	15 DAS	30 DAS	35 DAS	45 DAS	65 DAS
Limonene	60.5 e	68.2 d	72.0 c	79.0 a	74.0 b	74.0 a	74.0 a	74.0 a	74.0 a	72.0 b	71.0 c
Quercetin	1.3 de	1.4 d	1.6 b	2.7 a	1.5 c	1.5 a	1.5 a	1.5 a	1.5 a	1.4 b	1.2 c
Perillyl alcohol	1.1 e	1.2 d	1.3 c	3.5 a	1.4 b	1.4 b	1.4 a	1.4 a	1.4 a	1.3 b	1.1 c
Monoterpenes	1.4 e	1.5 d	1.6 c	2.8 a	1.7 b	1.7 b	1.7 a	1.7 a	1.7 a	1.5 b	1.3 c

The results are presented as mean based on $n=3$, *LSD* Least significant differences at ($p<0.05$). Different letters within column indicate significant differences between harvesting times and storage periods ($p<0.05$)

Table 4 Means of flavonoids compounds in grapefruit (*Citrus × paradisi*) cv Shamber at Storage periods and various harvested dates (mg/100 g)

Flavonoids (mg/100 g)	Harvesting dates					Storage periods					
	Sep. 1st	Oct. 1st	Nov. 1st	Dec. 1st	Jan. 1st	0 DAS	15 DAS	30 DAS	35 DAS	45 DAS	65 DAS
Hesperidin	2	3	4	6	5	5	5	5	5	4	3.5
Hesperitin	0.1	0.2	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.2	0.1
Narirutin	40	50	60	90	88	88	88	88	88	86	85
Natsudaaidain	—	—	—	—	—	—	—	—	—	—	—
Neohesperidin	6	7	11	14	12	12	12	12	12	11	10
Neoponcirin	—	—	—	—	—	—	—	—	—	—	—
Nobiletin	1.1	1.3	1.4	1.6	1.4	1.4	1.4	1.4	1.4	1.3	1.2
Poncirin	1.4	2.6	3.6	10.6	9.1	9.1	9.1	9.1	9.1	8.6	8.5
Rhoifolin	1.2	2.2	3.4	4.7	3.3	3.3	3.3	3.3	3.3	3.2	3.1
Rutin	1.1	1.2	1.4	2	1.9	1.9	1.9	1.9	1.9	1.6	1.5
Taxifoli	1.2	2.2	3.4	7.8	6.5	6.5	6.5	6.5	6.5	6.4	6.2
Gallic	2.2	3.4	7.8	6.5	3.1	3.1	3.1	3.1	3.1	2.9	2.7

The results are presented as mean based on $n=3$, *LSD* Least significant difference ($p < 0.05$)

Fig. 1 The chorogram of four terpenoid compounds in juice of grapefruit with peaks numbers were (1) Limonene (2) Quercetin (3) Perillyl alcohol (4) Monoterpenes

as compared to other harvesting dates. In case of other compounds, i.e., Poncirin, Rhoifolin Rutin, and Taxifolin also reflect the same trend during the season; increases from early to late in the season (Table 4). However the all compounds showed little reduction in 45 and 65 days of storage periods. The chorogram of all 12 compounds were shown in Fig. 2.

Discussion

The harvested times and storability of *Citrus paradisi* (grapefruit) cv. Shamber examined in this study reflected that commercial harvest time starts with early winter (November and December) in agro-climatic regions “Sargodha,” Pakistan. The current study to design the compare of storage and seasonal changes on essential compounds. The storage with suitable temperature and humidity is crucial factor to stable

Fig. 2 The chorogram of ten flavonoids compounds in juice of grapefruit was detected while two compounds were un detected with numerical numbers as peak of all compounds were (1) Hesperidin (2) Hesperitin (3) Narirutin (4) compounds was not detected Natsudaaidain, (5) Neohesperidin (6) compounds was not detected Neoponcirin (7) Nobiletin (8) Poncirin (9) Rhoifolin (10) Rutin (11) Taxifoli (12) Gallic

the compounds stability, the largest fruit crops of the world *Citrus* fruits, generally known to have a poor shelf life with problems related to the post and pre-harvested losses due to the seasonal changes and more health compounds have been losses in storage periods [2]. The total sugar, fructose, glucose and sucrose were varied significantly during different maturity stages of the season (Table 1). The similar results were found in other studies as reported by Pereira et al. [10] 10 to 12 mg⁻¹ 100 g FW in cv. Ray ruby. The total sugars increased, and total acids decreased from early to late in the seasons (Table 1) may be the consequences of growth and maturity periods, which was similar to the work of Yousef et al. [11]. In storage periods the sugar contents

were increased in all stored days and all individual sugars were constantly increased the similar finding of Liu et al. [12] also reported that the increased level of sugars might have due to the consequence of starch and hemicellulose hydrolysis process in storage periods. The phenolic acids are the well-known class of plant secondary metabolites, very effective free radical scavengers show multiple medicinal and biological functions in animals as well as in plants [13]. The early and late harvested periods showed lower values due to developmental stages, the reduction of substrate supply may affect the biosynthesis of phenolic acids speculated that the presence of sucrose is necessary for polyphenol biosynthesis during the growth season process. Storage periods of fruits (35) days have constant values of phenolic acids due to its the conversion of various enzymatic changes and Phenolic acids in plants primarily derived from the phenylpropanoid biosynthetic pathway was started [14]. The lower values of storage periods at 65 day due to the PAL functions at the entry point of the phenolic acid pathway by catalyzing phenylalanine to cinnamic acid was done in stored fruits [6]. The results of the present study are similar to previous studies. The lower levels of phenolic contents during early growth and development stages might also be explained with the concept as demonstrated by other researchers, reaching a minimum when soluble solids begin to accumulate, the changes were observed. The initial decrease could be, in part, due to the decrease in tannin content, as found in other studies [10]. The terepene are major class of phytochemicals in grapefruit juice for its taste and favour the early and late harvesting dates are decreased the contents due to particular antioxidants can break and seize free radical chain reaction during oxidation and form stable free radicals, which would not initiate or spread further oxidation process. The increased level of terpene in December presented higher antioxidant activity as compared to fruit harvested in early in the season, after that, the levels of phenolic compounds and total antioxidants capacity increased significantly with maturation, process due to changes in cell wall and its metabolites similar to the other finding. Terepene are lower in 65 days of storage periods due to sugar and acidity should be reduced in juice as pervious reported of Ahmed et al. [2]. Flavor is complex mechanisms for the breakdown of different bitter flavonoids and tannins in stored periods as reported by Duthie and Crozier. The contents of different flavonoids in the present study (Table 4) are in accordance with the previous studies reported in chlorogenic acid in sweet oranges and gallic acid in white grapefruit [6]. Grapefruit and oranges are rich sources of phytochemicals have different ranges of p-coumaric acid 21–25 mg/100 g. Yousef et al. [11] reported that the effect of maturity on physicochemical properties of nectarine fruits have been reported in Barbara et al. conducted an experiment on blueberries for seasonal changes of bioactive compounds and found highest contents

of bioactive compounds in the late-harvested fruits of blueberries similar to the results of the current study (Table 4). Storage period on flavonoids are constant at 35 day of storage period while little reduction have been noted in 65 day of storage period shown in Table 4 the flavonoid are co linked with carotenoids.

Conclusion

The current study to design the compare of storage potential and seasonal variation on essential compounds. The harvesting times and storage periods are co-linked with minimized the loss of essential compounds in grapefruit juice at storage and harvesting times. The early and late harvesting time showed lower level of all compounds while prolonged storage also reduction of these compounds. The sugars profiling, phenolic acids, 12 essential flavonoids, and terpenes significantly increased in late-harvested fruits as compared to early harvested fruits. However in storage periods of 45 and 65 days all these compounds had some lower values were noted, It can be concluded that the December harvested time and 35 day of storage periods have full the nutritional values while in comparison of early and late harvested periods. Furthermore, the data reinforce the importance of grapefruit intake to provide antioxidants, with range of polyphenol, the best time for consuming the grapefruit in month of December have potential health related compounds.

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Author contributions WA contributed in collecting plant sample, identification and herbarium confection. Conceived and designer the experiments: WA, RA. Performed the experiments RA, AQ, AM, ML. Analyzed the data: WA, AQ, MA, AM, SMK. Wrote the paper: WA, RA, ML, and SMK. All the authors have read the final manuscript and approved the submission.

Compliance with ethical standards

Conflict of interest No conflict of interest among the authors and co-author.

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References

1. W. Ahmed, R. Azmat, S.M. Khan, M.S. Khan, A. Qayyum, A. Mehmood, S.U. Khan, I. Hussain, Pharmacological studies of *Adhatoda vasica* and *Calotropis procera* as resource of bioactive compounds for various diseases. *Pak J of Pharm Sci* **31**(5), 1975–1983 (2018a)
2. W. Ahmed, R. Azmat, S.M. Khan, M.S. Khan, A. Qayyum, A. Mehmood, S.U. Khan, I. Hussain, Pharmacological studies of isolated compounds from *Adhatoda vasica* and *Calotropis procera* as an antioxidant and antimicrobial bioactive sources. *Pak J of Botany* **50**(6), 2363–2367 (2018b)
3. T. Albishi, J.A. John, A.S. Al-Khalifa, F. Shahidi, Antioxidant, anti-inflammatory and DNA scission inhibitory activities of phenolic compounds in selected onion and potato varieties. *J. Funct. Foods* **5**, 930–939 (2013)
4. P. Buachan, L. Chularojmontri, S.K. Wattanapitayakul, Fruits on human endothelial cells: Enhancing cell migration and delaying cellular aging. Selected activities of citrus maxima merr. *Fruit Nutr* **6**, 1618–1634 (2014)
5. Z.T. Chen, H.L. Chu, C.C. Chyau, C.C. Chu, P.D. Duh, Protective effects of sweet orange (*Citrus sinensis*) peel and their bioactive compounds on oxidative stress. *Food Chem.* **135**, 2119–2127 (2012)
6. W. Ahmed, R. Azmat, S.M. Khan, M.S. Khan, A. Qayyum, A. Mehmood, S.U. Khan, The role of chitosan to prolonged the fresh fruit quality during storage of grapefruit CV. Ray ruby. *Pak J of Botany* **50**(1), 151–159 (2018c)
7. S. Sankhalkar, V. Vernekar, Quantitative and qualitative analysis of phenolic and flavonoid content in *Moringa oleifera* lam and *Ocimum tenuiflorum* L. *Pharm. Res.* **8**, 16 (2016)
8. AOAC, *Association of Official Analytical Chemists International Official Methods of Analysis*, 16th edn. (AOAC, Arlington, 1997)
9. J.F. Williams, R.G. Muttters, C.A. Greer, Rice nutrient management in California. The Regents of the University of California, Agriculture and Natural Resources, Publication 351 (2010)
10. R.P. Pereira, A.A. Boligon, A.S. Appel, R. Fachineto, C.S. Ceron, J.E. Tanus-Santos, M.L. Athayde, J.T. Rocha, Chemical composition, antioxidant and anticholinesterase activity of *Melissa officinalis*. *Ind. Crop. Prod.* **53**, 34–45 (2014)
11. S. Yousef, A. Ghorban, H. Nematzadeh, I. Mohammad, Influence of harvesting date on some physicochemical properties of nectarine leaf and fruit. *J Med Plants Res* **6**(43), 5552–5556 (2012)
12. E.H. Liu, P. Zhao, L. Duan, G.D. Zheng, L. Guo, H. Yang, P. Li, Simultaneous determination of six bioactive flavonoids in *Citrus Reticulatae* Pericarpium by rapid resolution liquid chromatography coupled with triple quadrupled electrospray tandem mass spectrometry. *Food Chem.* **141**, 3977–3988 (2013)
13. K. Ghasemi, Y. Ghasemi, M.A. Ebrahimzadeh, Antioxidant activity, phenol and flavonoid contents of 13 Citrus specis peels and tissues. *Pak. J. Pharm. Sci.* **22**(3), 277–281 (2009)
14. J. Zhang, M. Tang, L. Viikari, Xylans inhibit enzymatic hydrolysis of lignocellulosic materials by cellulases. *Bioresour. Technol.* **121**, 8–12 (2012)

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