COMPARATIVE EVALUATION OF TOTAL IRON, VITAMIN C AND TOTAL PHENOLIC CONTENTS IN FRESH JUICES OF APPLE (*MALUS PUMILLA*) GROWN IN PAKISTAN.

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خلاصہ

فینولک مرکبات کا انسانی صحت پر بہت اچھا اثر ہوتا ہے۔ کیونکہ یہ اینٹی پرولیفیریٹو اور اینٹی آکسیڈینٹ ہے۔ وٹامن سی غذا کا ایک اہم جز ہے یہ بھی اینٹی آکسیڈینٹ اور تھیراپیوٹک خصوصیات کا حامل ہے۔ فولاد بھی انسانی جسم میں ھیموگلوبن کو توازن میں رکھنے کے لئے درکار ہوتا ہے۔ پھلوں اور سبزیوں میں یہ مرکبات بڑی مقدار میں موجود ہوتے ہیں۔ اس کام کا مقصد پاکستان کے مختلف علاقوں میں کاشت کئے گئے سیبوں کے جوسز کی طبعی و کیمیائی خصوصیات پی ایچ، کنڈیکٹویٹی اور ٹوٹل حل پذیر ٹھوس کا مطالعہ اوران جوسز میں فینولک مرکبات، وٹامن سی اور فولاد کا معلوم کرنا شامل ہے۔ پی ایچ، کنڈیکٹوٹی اور ٹوٹل حل پذیر ٹھوس کی قیمتیں بل ترتیب درج ذیل ہیں 3.858–4.100, 2.04–2.53 mS cm^{-1}اور 1201–1531 mg/100 mL جبکہ فولاد اور وٹامن سی کی تخمین کے لئے کلاسیکل طریقہ کار استعمال کیا گیا انکی قیمتیں بلترتیب یہ ہیں۔ 1.076± 0.02 – 1.688 ± 0.02 mg/1000 mL اور 8.79±0.55 – 45.56 ± 1.05 mg/1000 mL جبکہ ٹوٹل فینولک مرکبات کی تخمین بذریعہ ایف سی متعامل اسپیکٹروفوٹومیٹر استعمال کرکے حاصل کی گئی۔ ان کی مقدار یہ پائی گئی۔ 40.8 ± 1.30 – 59.8± 1.05 mg/1000 mL جب ان کا موازنہ شائع شدہ قیمتوں سے کیا گیا تو مشاہدہ میں آیا کہ پاکستان میں کاشت کئے گئے سیبوں میں یہ مرکبات زیادہ مقدار میں پائے جاتے ہیں لہذا یہ انسانی جسم میں ان مرکبات کی روزانہ ضراریات کو پورا کرنے کا اہم ذریعہ ہیں۔

Abstract

Phenolic compounds have a great impact over human health because of their antioxidant and antiproliferative properties. Vitamin C is also a valuable food component having antioxidant and therapeutic properties and iron is the essential element required to maintain the hemoglobin level in human beings.Fruits and vegetables are rich sources of these compounds. The purpose of this work was to study physiochemical properties like pH, conductivity and total dissolve solids and to find out the amounts of total iron (ferrous and ferric), vitamin C and total phenolic contents in fresh juices of apples cultivated in different areas of Pakistan.The values of pH , conductivity and total dissolved solids were recorded in the range of 3.858–4.100, 2.04–2 .53 mS $cm^{-1}$and 1201–1531 mg/100 mL respectively. Classical method of analysis was applied to investigate the iron and vitamin C content , it was found as 1.076± 0.02 –1 .688± 0.02 mg/1000 mL and 8.79± 0.55– 45 .56±1.05 mg/1000 mL respectively . The range of total phenolic content was observed from 40.8 ± 1.30 –59 .8± 1.05 mg/1000 mL of juice using Folin Ciocalteu reagent by spectrophotometer. It was found that obtained results exhibited higher concentration of iron, vitamin C and total phenolic contents in fresh samplejuices than reported data therefore, it is concluded that apples grown in Pakistan have remarkable aggregate of iron, vitamin C and total phenolic contents and play crucial role to serve the human daily requirement.

Key words: Apple juices, vitamin C, total phenolic contents, iodimetry and spectrophotometry.

Introduction

Apple *(Pyrus malus L*) belongs to the family Rosaceae and sub-family pomoideae, it is one of the main fruit crops of the world (Chaudhary, 1994). Apples are cultivated in Pakistanin most of the hilly areas of Baluchistan, KPK and Gilgit Baltistan because of the fertility of soil and most favorable environmental conditions (Ali *et al*., 2011).Temperature and environmental variations have an imperative effect on food production like color, flowering time, and other morphological characters of the fruit. It also influences the flavor and other nutrients of apple. (Slingo, 2009).

Apples are taken as a delicious fruit but these are also used in different food items like desserts, jams and in drinks. It also shows noticeable changes on health. (Sandor, 2008). Vitamin C is the most significant Vitamin for human nourishment and it is provided by fruits and vegetables because of its antioxidant and therapeutic properties (Okiei *et al*., 2009).

Various studies have established that fruits and vegetables improve a protective strength against the development of human diseases such as heart oriented problems, diabetes and cancer (Hu, 2003). Apple

contains noticeable concentration of phenolic compounds and most of these exhibit relevant antioxidant properties in vitro (Boyer and Liu, 2004). Many researches have made a strongbond between antioxidant potential bearing compounds (polyphenolic) and reduced option of numerous diseases and it has the ability to bring down cellular damage and can be favorable in supporting human health against cardiovascular and respiratory disorders, cancers and diabetes. (Hollman and Katan, 1999; Fu *et al*., 2011; Hyson, 2011).It is also reported by Emberhardt (2000), that fresh apples inhibit the growth of different cancer cells because of these total phenolic contents.

Iron is present in fruits and vegetables in noticeable quantities (Hurrell and Egli, 2010). In the human body, iron is a key part of hemoprotein like hemoglobin and myoglobin, it also plays asignificantpart as a ferritin (Mc Dowell, 2003). According to Hurrell (1997), iron is necessary for the enzymes required in electron transfer and other oxidation reduction reactions in human body.

Materials and Methods

Apple samples were collected in the first week of September 2018. These samples were cultivated in Quetta (Balochistan), Swat (Khyber Pakhtun Khuwa) and Bagrot (Gilgit Baltistan) and were labelled in this manner QB, SK and BGB respectively.

The samples were washed with tap water, rinsed with distilled water and dried in open air. After weighing on analytical balance(Denver instrument, TP 214, 25160149), the juice was extracted by using electric juice extractor (Philips HR 1823/70) and preserved in plastic bottles and kept these in freezer at ─ 18 ℃. All the chemicals used in this work were of analytical grade.

The pH of each samples was determined by immersing the electrode of pH meter (Jenway 3510) in sample juices and conductivity and TDS (total dissolved solids) were noted by using conductivity meter (Jenway 4510). Concentration of iron was estimated by the method described in Mahmood *et al.,* 2006.

The vitamin C content was determined by classical mode of examination based on iodimetry (Redox reaction) in which sample juices were reacted with standard iodine solution in the presence of starch as an indicator following Silva *et al*., 1999.

Total phenolic contentswere carried out by using Folin-Ciocalteu method as described by Skerget *et al*., (2005).Absorbance of these solutions were measured at 765 nm using (721-VIS) spectrophotometer. These results were exhibited as mg gallic acid equivalents per 100 mL samples.

Results and Discussion

The pH values detected in apple juice samples were in between 3.858 to 4.100(Table 1) SK sample showed the lowest pH value and BGB has the maximum value of pH, these are the same values as Jan *et al.,* (2016) has reported. According to Rahmanian *et al.*, 2015, conductivity is the way to measure the presence of minerals in the samples and these minerals play important roles as electrolytes in the human body. Sample BGB contained minimum concentration of minerals, 2.04 Ms cm^{-1}, SK and QB showed 2.44 and 2.53 mS cm^{-1}respectively. It means that QB contained maximum value of electrolytes and it can be a good source of required electrolytes.

Total dissolved solids were recorded 1201 for SK, 1531 and 1460 for QB and BGB respectively, The maximum permissible limit of total dissolved solids in drinking water according to PSQCA (Pakistan Standards & Quality Control Authority), is 1000 mg/L (Mahmood, S. *et al.*, 2013).Increased values of TDS in our samples, is due to the presence of metal ions which are necessary for proper and balanced human health.

Total iron concentration in these samples was determined from 1.0776 ± 0.02 to 1.688 ± 0.02 mg /1000 mL (Table 2), BGB showed maximum concentration of iron, QB and SK showed 1.0776 ± 0.02 and 1.206 ± 0.01 mg /1000 mL (Table 2) respectively. When these values were compared with the values of Mahmood T *et al.,* 2013, it was concluded that our samples were iron rich and inter acting factors might be the responsible for this discrepancy.

The results given in Table 2 reveal the vitamin C values ranging from 8.79 ± 0.55 to 45.70± 1.05 mg/1000 mLin samples. It can be observed that QB has the lowest concentration among these samples and apples from KPK and Gilgit Baltistan, (SK and BGB respectively) have more values of vitamin C than results observed by Mahdavi *etal.* (2010). According to Mahdavi vitamin C in fresh apple juice was 17.45 mg/1000 mL. There is a great change in vitamin C content of these samples, especially GBG showed 45.70 ± 1.05 mg /1000 mL, it may be due to the difference in varieties and maturity states of fruits.

According to Mohammad *et al*., (2014) ripeness of fruit also affects the concentration of vitamin C, he reported that half ripe apples contain more vitamin C than full ripe apples, current studies supports his report since it was observed at the time of collection that the sample BGB was half ripe and its vitamin C value also justifies it.Methodsof investigation of this content and juice extraction process may also influence the vitamin C content of fruit juices (Gil-Izquierdo *et al*., 2002) thus way of handling, applied procedure andstorage modes of fruit cannot be ignored to explain these variations in results.

Table 2 also exhibits the obtained results of total phenolic contents in which BGB has a little difference and samples collected from KPK and Balochistan (SK and QB respectively) indicate the significantly higher concentration of total phenolic compounds as compared to reported values (45.3 mg /1000 mL) of Mahdavi *etal.* (2010). The values of total phenolic contents can be influenced by the climatic factors of growing areas because these samples were collected from three different places of Pakistan, (Balochistan, Gilgat Baltistan and Khaiber Pukhtoonkhua) and these places show a great diversity in their cultivation modes, nature of soil and source of water supplied. Muhammad *et al*., (2014) reported that these inter acting factors have an unlimited effect on the quality parameters and biochemical composition of fruittherefore it can be reported that these elements are responsible for change in concentration of total phenolic contents in these apple samples.

Table 1: **Physio-chemical properties of apple juice samples.**

Sample code	Volume of juice (mL/100g)	pH	Total Dissolve Solids (TDS) mg/100mL	Conductivity $mScm^{-1}$
QB	56.29	3.956	1531	2.53
SK	44.76	3.858	1201	2.44
BGB	38.46	4.100	1460	2.04

QB=Quetta (Balochistan) SK=Swat (Khyber Pakhtun Khuwa) BGB=Bagrot (Gilgit Baltistan)

Table 2: **Values of vitamin C and total phenolic contents in different apple juice samples.**

Sample code	Total Iron Mean ± SD (mg/1000mL)	Vitamin C Mean ± SD mg/1000mL	Total phenolic contents Mean ± SD mgGAE/1000Ml
QB	1.076± 0.02	8.79± 0.55	53.80± 1.20
SK	1.206± 0.01	45.56± 1.05	59.80± 1.05
BGB	1.688± 0.02	28.59± 0.92	40.80± 1.30

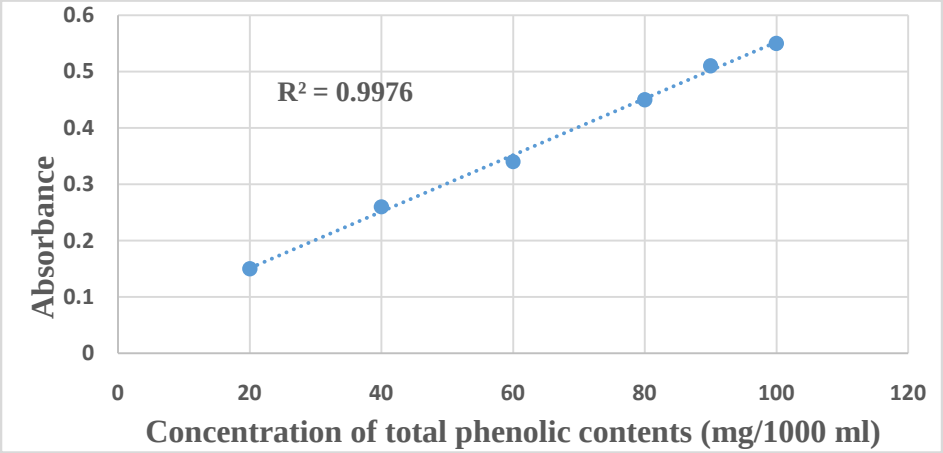

Fig 1: **Calibration curve of Total phenolic contents.**

Conclusion

It is concluded that apples of QB (Quetta, Balochistan) are good on the basis of physio-chemical properties, these had much volume of juice per 100 gram of sample and it contained more total dissolved solids and minerals concentration than other samples. The samples of SK (Swat, Khyber Pakhtun Khuwa) had proven itself a great source of total phenolic and vitamin C contents but the samples of BGB (Bagrot, Gilgit Baltistan) cannot be ignored because these were more rich in iron than both other samples.

In the light of above work it is concluded thatapples grown in different areas of Pakistan are rich in minerals, iron, vitamin C and total phenolic contents and these are necessary for proper health and active life.Vitamin C is one of the most significant antioxidant vitamins and supports to keep body tissue healthy, makes immune system more efficient and helps to absorb iron from plant sources. Therefore we should maintain the level of these compounds in body according to the recommended daily intake. It is experienced that

apples are easily available in markets from September till April at very reasonable rates so people should eat it and drink its juice instead of other soft drinks.

References

Ali M., Ayub M., Zeb A., Durrani Y., Ullah J. and Afridi S. R.(2011). Physicochemical analysis of apple pulp from Mashaday variety during storage. *Agric. Biol. J. N. Am.*, 2: 192-196.

Boyer J. and Liu R. H. (2004). Apple Phytochemicals and Their Health Benefits. *Nutrition Journal*, 12, 3-5.

Chaudhary, M.I.(1994). Fruit crops. In: *Horticulture.* National Book Foundation, Islamabad, Pakistan, 468.

Emberhardt M., Lee C. and LiuR. H. (2000). Antioxidant activity of fresh apples. *Nature*, 405: 903-904.

Fu, L., Xu B. T., Xu X. R., Gan R. Y., Zhang Y., Xia E. Q. and Li, H. B. (2011). Antioxidant capacities and total phenolic contents of 62 fruits. *Food Chemistry*, 129:345-350.

Gil-Izquierdo, A., Gil M.I. and Ferreres F.(2002). Effect of processing techniques at industrial scale on orange juice antioxidant and beneficial health compounds. *J. Agric. Food Chem.*, 50:5107-5114.

Hollman P. C. H. and Katan M. B. (1999). Dietary flavonoids: Intake, health effects and bioavailability. *Food and Chemical Toxicology*,37: 937-942.

Hu F.B.(2003). Plant-Based Foods and Prevention of Cardiovascular Disease: An Overview. *The American Journal of Clinical Nutrition*, 8: 544S-551S.

Hurrell R. and Egli I. (2010). Iron bioavailability and dietary reference values. *American Journal of Clinical Nutrition*. 91:1461-7S.

Hurrell R. F., (1997). Bioavailability of iron. *European Journal of Clinical Nutrition,* 51:S4-8.

Hyson D. A. (2011). A comprehensive review of apples and apple components and their relationship to human health.*American Society for Nutrition:* Advance Nutrition, 2: 408-420.

Jan A., Amir G., Raees H., Amir P., Shaista T. and Tariq A. S. (2016).Physico-Chemical Analysis of Apple Juice Concentrates from Kashmir Valley. *American Journal of Food and Science Nutrition Research* 3(3): 42-45.

Mahdavi R, Nikniaz Z., Rafraf M. and Jouyban A. (2010). Determination and Comparison of Total Polyphenol and Vitamin C Contents of Natural Fresh and Commercial Fruit Juices. *Pakistan Journal of Nutrition* 9: (10) 968-972.

Mahmood S., Asif A., Awaar A., Nauman K. and Tariq J.(2013). Drinking Water Quality in Capital Cities of Pakistan. *Open Access Scientific Reports*. 2(2), 637 doi:10.4172/ scientificreports.637.

Mahmood T., Aiman M., Humaira A., Iffat M. and Aneela W. (2013). Quantification of total iron content of malus pumilla (apple) using spectrophotometric method. *FUUAST J. Biol.* 3(1): 75-78.

Mahmood, S. Z., Arshad H. S. and Arshia S. (2006). *Amali Chemia, Inorganic and General*.Edara Tasneef o Taleef o Tarjuma, FUUAST, Karachi.

McDowell L.R., (2003).*Minerals in Animal and Human Nutrition*. 2nd ed. Amsterdam: Elsevier Science; p. 660.

MuhammadI., Ashiru S., Ibrahim I. D., Kanoma A. I., Sani I. and Garba S.(2014). Effect of ripening stage on vitamin C content in selected fruits. *International Journal of Agriculture, Forestry and Fisheries*, 2: (3) 60-65.

Okiei, W., Ogunlesi M.,Azeez L., Obakachi V., Osunsanmi M. and Nkenchor G. (2009). The voltametric and titrimetric determination of ascorbic acid levels in tropical fruits samples. *Int. Journal of electro chemistry, Sci*., 4:276 – 287.

Rahmanian, N., Ali, S.H.,Homayoonfard, M., Ali, N.J., Rehan, M., Sadaf, Y. and Nizami, A.S. (2015). Analysis of Physiochemical Parameters to Evaluate the Drinking Water Quality in the State of Perak, *Malaysia. Journal of Chemistry*. 2015:10.

Sandor, F. (2008). Apple Production. Perennial Crop Support Series Jalalabad, Afghanistan. Manual produced by Roots of Peace, USAID, Afghanistan, California. Alternative Livelihood Program-Eastern Region ALP/E. Publication No. 2008-004-AFG.

Silva R. S., SimoniJ. A., CollinsH. C. and Volpe O. L. P. (1999). Ascorbic acid as a standard for iodometric titrations. An Analytical Experiment for General Chemistry.*J. Chem. Educ.*, 76: (10), 1421-1422.

Singleton, V.L., Joseph A. and RossiJ.R.J.A. (1965). Calometry of total phenolic with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticulture*, 16: 144-158.

Skerget, M., Kotnik P., Hadolin M.,Hraš A. R., Simonič M. and Knez, Z. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry* 89: 191– 198.

Slingo, M. (2009). Effect of climate change on apple production in New Zealand. Ter. Ecosys. Interact .Global. Changes. 2: 673-687.