Comparative Study of Two Pear (Pyrus communis L.) Cultivars in Terms of Nutritional Composition

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Abstract
Nutritional composition of two pear cultivars (Pyrus communis) was analyzed for sugar composition, amino acid, and fatty acid profile by using high-performance liquid chromatography (HPLC) equipped with RI and ultraviolet detectors and GC equipped with FID detector. Proximate composition was determined by using the standard methods of AOAC (2000). The results revealed that the Shughri contain 83.1% moisture content, 13.58% TSS, 3.94% ash content, 11.72% crude protein, 9.47% crude fiber, 3.39% lipids and 96.46% carbohydrates, while Physhu contains less amounts of moisture, TSS, ash, protein, fiber, lipids and carbohydrates (54.51, 13.71, 1.86, 8.81, 7.83, 2.14 and 87.67% respectively). The results further revealed that the fructose was the major sugar in both pear cultivars, followed by glucose and sucrose. Linoleic acid (C18:2), palmitic acid (C16:0) oleic acid (C18:1) and α-linoleic acid (C18:3) were most abundant fatty acids found in both cultivars. Among essential amino acids leucine, lysine and isoleucine are most prominent in both cultivars. Among non-essential amino acids aspartic acid and glutamine are abundant. Anti-nutritional content analysis revealed that hydrocyanic, nitrate, oxalate and phytate were in lower amounts than the reference toxic standards. Thus, both pear cultivars have potential nutraceutical uses. The findings of this investigation provide important information on how to make the best use of pear cultivars studied for different uses, which is meaningful for both processing practices and technological research.

Keywords: Comparative study, Nutritional composition, Pyrus communis, Anti-nutritive compounds

1. Introduction
Pear fruit (Pyrus communis L.) belongs to the family Rosaceae, subfamily Maloideae or Spiraeoideae and genus Pyrus L. Fresh fruits an important ingredient of the diet and are generally regarded as healthy foods. Fruits are key sources of vitamins such as ascorbic acid and folic acid as well as a good source of dietary fiber (Brian and Cameron, 1995). Plant origin foods are capable of contributing considerable amounts of nutrients, such as protein, minerals, amino acids and other functional ingredients required for all age group of people, if properly processed and blended (Okaka et al., 2002). Pears are native to coastal and temperate zones of Western Europe and North Africa. The tree of pear fruit is medium sized in height, about 10 to 17 meters tall. Leaves are alternately arranged, simple, 2 to 12cm long; the flowers are white in color, 2 to 4cm in diameter. The pear fruit diameter is 1 to 4 (Brian and Cameron, 1995). Pears fruit is an excellent source of vitamin C, less allergenic than many other fruits and its juice is sometimes used as the first juice introduced to infants (Vadivel and Janardhanam, 2005). Pears are consumed fresh and canned. The nutritional importance of p. communis is not well known among the majority of consumers of these fruits.

The world largest pear producing country is People's Republic of China in 2013 with total production of 16266000 tonnes followed by United state of America with 77858 tonnes, Argentina with 70000 tonnes and Italy with 645540 tonnes, while Turkey, Spain, Republic of Korea, India, South Africa and Japan are the 4th, 5th, 6th, 7th, 8th, 9th and 10th largest per fruit producing countries (FOASTAT, 2013). Currently, the cultivation of the pear is incessantly rising throughout the globe particularly in Asia (Fischer and Weber, 2005). Pear is among the most admired pome fruit of Pakistan. The total production of this fruit is about 24152 tons per year (Anon. 2008). Annual production of pear in Gilgit-Baltistan is 2579 tons which is mostly produced in mountainous areas such as Hunza-Nagar district, Bagrote, Haramosh valleys of Gilgit region (Hussain et al., 2013).

Pear fruit is rich in macronutrients as well as in micronutrients (Senser et al., 1999). It is an excellent source of dietary fiber and also a good source of vitamin C (Silas et al., 2003). It contains a higher percentage of dietary fiber as compared to other fruits and vegetables. Dietary fiber content of this fruit can be possibly seen as a potent food additive (McKee and Latner, 2000). A medium sized fresh pear weighing 100g contains 15.46g carbohydrates, 9.80g sugars, 0.38g protein, 0.12 g fat, 83.71g water, 119.00 mg potassium, 4.20mg vitamin C, 11.00mg magnesium, 7.00mg iron and 0.10mg zinc (USD4, 2011). Pear fruit also contains health promoting bioactive compounds such as carotenoids (anthocyanins, flavanols, quercetin, kaempferol, isorhamnetin) and plant sterols (Andreotti et al., 2006). The fruit also contains a wide range of phenolic compounds comprising...
different flavonoid classes (chlorogenic, syringic, ferulic and coumaric acids, arbutin and (-)-epicatechin, hydroxyphenolic acids and the p-hydroquinone-glucoside arbutin) (Schieber et al., 2001; Petkou et al., 2002; Salta et al., 2010).

Interest in pear fruit in the world is increasing due to its numerous health benefits and rich nutritional profile. Pear fruit from this part of the world has not been investigated for their compositional information. Therefore, the data generated would be of great value for postharvest technology and also for consumer awareness. Determining physico-chemical properties of fruit will be important for the designing of equipment for harvesting, transporting, sorting, cleaning and packaging etc (Ozturk et al., 2009). It further assists to determine the maturity or harvesting time and evaluation of product quality. No previous data is available regarding nutritional prospective of pear fruit grown in this high-altitude region. Therefore, this paper was intended to determine the nutritional characteristics of some of commonly grown pear cultivars to produce firsthand information for producers, scientists, marketing entrepreneurs as well as for the end consumers.

2. Materials and Methods
2.1. Sample Collection
The pear fruits locally called Shughri and Physhu were purchased from a local fruit market of Gilgit-Baltistan province of Pakistan. Fruits were transported to Department of Food Technology PMAS-AAUR, Rawalpindi, Pakistan in polyethylene bags to reduce moisture loss during transportation. After arrival at the laboratory of the fruits were cleaned, graded and packed in polyethylene bags and kept under refrigeration until further analysis.

2.2. Proximate Analysis
The proximate analysis of fruits was determined in triplicates according to the standard methods of AOAC (2000). Nitrogen content was converted to protein using a factor 6.25 (Sotelo et al., 1995). Carbohydrate content was calculated by difference.

Moisture content was determined by the methods described by AOAC (2000). A dried and clean crucible was placed in oven for one hour, cooled in a desiccator and weighted. One gram (1g) of the fresh sample was weighed into a pre-weighed crucible and placed into a hot drying oven at 105°C for 24 hours. The sample was removed, cooled and placed in a desiccator for some time and weighed again the process was repeated until a constant weight was obtained.

\[
\text{Moisture Content (\%) = \frac{\text{Loss in wt. of sample (g)}}{\text{Wt. of sample (g)}} \times 100}
\]

2.3. Sugar Analysis
Composition of sugars was determined by following the method described by Dolenc and Stampar (Dolenc and Stampar, 1997), with slight modifications. Fruit samples were prepared by using four to five fresh fruits from each cultivar. 10 ml of fruit juice was diluted to 100 ml with distilled water, and then filtered through a 0.45 µm Millipore filter. 20 µl of and an aliquot was injected into the HPLC (KNAUER, Berlin, Germany). The mobile phase consisted acetonitrile-water (85:15, v/v), at a flow rate of 1.5 ml/min at 25°C.

2.4. Fatty Acid Analysis
The fatty acid profile was determined by using gas chromatography (GC) coupled with FID after esterification of the polar lipid (Pablo et al., 2000). 10 ml of sample juice from fruit samples were placed in tared test tubes, heated at 100°C for about five minutes to inactive the lipase, and then 30 ml methanol (1:2, v/v) was added to the sample. Samples were extracted for 25 minutes and filtered with 20 ml ethanol, and the resultant extracts was shaken in a vibrator (WZS-200A, Shanghai, China) for 15 minutes with 20 ml (w/v) NaCl (0.76%). The aqueous layer then was removed and the residual vaporized using a vacuum machine (RE-3000, Beijing, China), and was re-dissolved in 6 ml petroleum ether with saturated methanol and 6 ml methanol with saturated petroleum ether. The substrates were re-extracted with 6 ml of the same methanol and concentrated, and then dissolved in 1 ml GC grade methanol for storage at -20°C for process of esterification.

The polar lipid was re-dissolved in 2 ml of KOH (0.4M) in methanol and 2 ml benzeene-petroleum ether (1:1, v/v), vibrated for 30 minutes, and then allowed to rest for 15 min. Distilled water (20ml) was added to the vials, the upper layer of the extracts with-drawn and vaporized under pure nitrogen stream, and then re-dissolved in 1ml hexane for analysis by gas chromatography. Fatty acid analysis was done by using a HP Gas Chromatography (5890) machine with FID detector, connected to with BF-2000 chemstation software. Single aliquots of lipid extract (approximately 0.5µl) were injected in splitless mode onto the DB-23 (60m×0.25 mm I.D., 0.25 lm) column, (Agilent, USA). The temperatures of injector and detector were set at 250°C and 270°C, respectively. The flow rate was set at 32 cm/s, nitrogen was used as carrier gas, and the electronic pressure control set in the constant flow mode.
2.5. Analysis of Amino Acid

Five grams of the fresh sample from both cultivars were used to measure of nitrogen content by using the Kjeldahl method of AOAC (1990). Amino acids content in the samples was determined using ion exchange chromatography by Technicon Sequential multisampling Amino Acid Analyzer, using the method described by Hassan et al., (2009). 20mg of defatted flesh and peel samples were weighed into a glass ampoule to which 5 cm³ of HCl (6M) and 5 moles norleucine (2-amino hexanoic acid) internal standard was added. The ampoule was evacuated by passing nitrogen gas to avoid oxidation of some amino acids during the process hydrolysis. The ampoule was then sealed with a Bunsen burner flame stored in an oven thermostat at 105°C for overnight. The filtrate was then evaporated to dryness at 40°C under vacuum by using a rotary evaporator. The residue was then dissolved to 5µl and 10 µL for acid and neutral amino acids and for basic amino acids respectively. The aliquot was then loaded into cartridge of an amino acid analyzer. The chromatograms obtained along with automatic pen records indicate amino acids peaks corresponding to the magnitude of their respective concentrations. Quantification was determined by comparing the peak area of each amino acid in the sample to the area of the corresponding amino acid standard of the protein hydrolysate (0.02 µ mole).

2.6. Anti Nutritive Compounds Analysis

Anti-nutritive compounds found in both pear cultivars were determined by adopting the method of Hassan et al., (2009b).

2.7. Statistical Analysis

All analyses were performed in triplicate and the results were expressed as mean ± SD. Where appropriate, the results were expressed on a fresh weight basis. Analysis of variance (ANOVA) was performed to compare the sample analysis at 5% confidence level.

3. Results and Discussion

3.1. Proximate Composition

The results to pertaining proximate composition of two pear cultivars have been given in Table 1. The result revealed that the Shughri cultivar has significantly (p<0.05) higher moisture content of 52.3%, while the Physhu cultivar has a lower moisture content (54.51%). The higher moisture content in fruits and vegetables is associated with increase of microbial activities during storage (Hassan et al., 2009a; Mahammad et al., 2010). The moisture content of pear fruit is comparable with other fruits and vegetables (72-92%) (Hassan et al., 2009a;b; Mahammad et al., 2010). The TSS of both tested fruit genotypes ranged between 13.71 to 13.58 for Physhu and Shughri respectively. Low ash content and fiber reported in the present study is an indication of the sample to have low amount of mineral elements. Since ash content is an index of minerals present in the sample (Hassan et al., 2009a). Shughri has higher amounts of ash, crude fiber, lipids and carbohydrate (3.94, 9.47, 3.39 and 94.46%), while Physhu cultivar contain significantly (p<0.05) lower amounts of ash, crude protein, crude fiber, lipids and carbohydrate (1.86, 7.83, 2.14 and 87.67%). Proteins are essential component of diet which supplies adequate amounts of amino acids (Pugatenth i et al., 2004), protein deficiency causes growth retardation and abnormal swelling of the belly (Zarkada et al., 1997). The content of protein (11.72%) in Shughri is significantly (p<0.05) higher than Physhu cultivar (8.81%). Lipids are essential because they provide the body with maximum energy (Dreon et al., 1990). Higher fiber content may cause intestinal irritation and low digestively resulting in the decrease in nutrient utilization. Fiber helps in the maintenance of human health by reducing cholesterol level in the body and decreasing the risk of various cancers and improved general health (Hassan et al., 2009a). High carbohydrate content of feed is desirable while deficiency causes depletion of body tissue (Barker, 1996).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Proximate Composition (%)</th>
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<tbody>
<tr>
<td></td>
<td>Shughri</td>
</tr>
<tr>
<td>Moisture</td>
<td>83.1±2.13</td>
</tr>
<tr>
<td>TSS</td>
<td>13.58±0.34</td>
</tr>
<tr>
<td>Ash</td>
<td>3.94±0.04</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>11.72±0.12</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>9.47±0.1</td>
</tr>
<tr>
<td>Lipids</td>
<td>3.39±0.08</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>96.46±3.50</td>
</tr>
</tbody>
</table>
3.2. Amino acids Composition

The nutritive value of the plants protein quality is generally evaluated by comparing its essential amino acids content with reference standards set by (FOA, 1991), which is based on the amino acids need for the children aged 2 to 5 years. Essential and non-essential amino acid composition has been given in Figure 1 and 2. The major amino acids in both pear cultivars were glutamic acid and leucine. Shughri cultivar of pear contained significantly (p<0.05) higher amounts of all essential and non-essential amino acids. These amino acids were found to be higher than 1.9g/100g protein set as reference standard (FOA, 1991). This implies that the amino acids composition in tested pear fruit verities has a high biological value and could contribute in meeting the human requirements of these essential amino acids (Mahammad et al., 2010). The role of amino acids (mostly amino acids of aroma family) on fruit aroma has been previously reported in some studies. It has been reported that Tyrosine was the formation substrates for volatile compounds (Wang et al., 2002; Jiluan et al., 2007).

Figure 1. Essential amino acids found in two pear cultivars

![Essential Amino Acids](image1)

Figure 2. Non-essential amino acids found in two pear cultivars

![Non Essential Amino Acids](image2)

3.3. Fatty Acid Analysis

Fatty acid profile (%) of pear cultivars has been given in Figure 3, which indicated that the linoleic acids and plmitic were the principal fatty acids, constituting 70 to 80% of the total fatty acids in the pear fruit. According to the obtained results, the C18:2 (56.55 to 6.21) was the most abundant fatty acid followed by C16:0 (19.71 to 25.2), C18:1 (2.78 to 5.45), C18:3 (1.88 to 3.72), C18:0 (1.54 to 2.27), C16:1 (0.39 to 1.14), C12:1 (0.39 to 1.08), C17:0 (0.44 to 0.66), C20:0 (0.33 to 0.64) and C14:0 (0.15 to 0.56), while C20:1 and C20:4 was detected in
minor amounts in tested cultivars. Docosapentaenoic acid (C20:5) was not detected in both pear cultivars. The results were found significantly differed (p<0.05) among both tested pear cultivars. The cultivar Shughi had significantly (p<0.05) higher contents of all tested fatty acids.

The amounts of linoleic and α-linolenic acid in the plants are therefore significant, as both are indispensable fatty acids for humans and must be obtained through the diet (Jiluan et al., 2007). The function of fatty acids on fruit aroma has been investigated in earlier studies (Russell et al., 1981). For example, it has been studied that flavor compounds of Bartlett pear were synthesized when pear squash was incubated with C18-unsaturated oleic, linoleic, and linolenic fatty acids.

![Figure 3. Fatty acid profile of pear fruit](image)

### 3.4. Sugar Profile

In the current paper, we compared the soluble sugars composition of two pear cultivars. Recognition was established using identified standards. Fructose and glucose were recognized as the primary monosaccharides in both pear cultivars as shown in Fig. 4. The findings of this study indicated that fructose was the major sugar followed by glucose in both pear fruits cultivars. This accorded with the Code of practice for assessment of pear juices (AIJN, 2004). There have been many previous studies investigating fruit sugar and the increasing concentrations of fructose, glucose and sucrose at earlier fruit maturity stages (Jiluan et al., 2007; Chen and Yan, 2004; Gao et al., 2004). The results are given in Figure 4 shown that the fructose content of Shughri cultivar (74.52%) is significantly (p<0.05) higher than Physhu pear (51.18%). The amounts of glucose and fructose contents in the current study are in line with previously reported ranges of pear fruit (Chen and Yan, 2004; Gao et al., 2004). The sucrose content of Shughri cultivar (7.33%) is the significantly (p<0.05) higher than that of Physhu pear (3.77%). The sugar profile of fruits is a significant factor of chemical composition tables and provides important information regarding the authenticity of fruit juices, and individual sugar and total sugar contents correlated well with the sweetness characteristics of the fruit juice, based on sensory evaluation.

![Figure 4. Sugar composition of two pear cultivars](image)

### 3.5. Anti-nutritional Composition

The concentrations of the anti-nutritive compounds found in both pear cultivars are given Fig. 5 Shughi
cultivars contains 12.44% oxalate, 6.64% nitrate, 2.11% phytate and 1.08% hydrocyanic while on the other hand Phyu cultivars has 8.76% oxalate, 4.21% nitrate, 2.8% hydrocyanic and 1.33% phytate. The concentrations of above anti-nutritive compounds are under the recommended toxic levels (Birgitta and Caroline, 2000). Anti-nutritive compounds hinder with metabolic processes, so that growth and bioavailability of nutrients are negatively affected. These constituents stand as indices for judging the nutritional value of food substance (Binita and Khtarpaul, 1997). Oxalate can combine to calcium in food thereby interpretation calcium unavailable for normal physiological and biochemical functions. (Ladeji et al., 2004).

Figure 5. Anti-nutritive compound found in pear fruit

![Graph showing anti-nutritive compounds](image)

4. Conclusion
The current research work recommends that pear fruit is a nutritious fruit containing adequate amounts of essential nutrients. Pear fruit holds number of essential nutrient such as protein, carbohydrate and ash. Although no single plant food can offer with sufficient level of all nutrients requires by human being. Yet it provides with some essential amino acids, fatty acids and sugars moderate enough to meet with the recommended daily allowances. Additionally, pear fruit is rich in both essential and non-essential amino acid content. Moreover, certain anti-nutritive compounds were revealed such as phytate, nitrate, oxalate and tannins which are toxic and interfere with digestion and absorption, but all are under the toxic levels, therefore the fruit has low anti-nutritive constituents and it could serve as potential source in food formulation.

5. References
Abstract translated into English.
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